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SYMPOSIUM ON THE ANALYSIS OF CARCINO-
GENIC AIR POLLUTANTS HELD AT CINCINNATI,
OHIO ON AUGUST 30, 1961. VOLUME II

E. Sawicki, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

30 August 1961

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SYMPOSIUM

on

THE ANALYSIS OF CARCINOGENIC AIR POLLUTANTS

- - -

August 30, 1961

at

Cincinnati, Ohio

DR. E. SAWICKI, CHAIRMAN

- - -

Sponsored by the

LABORATORY OF ENGINEERING AND PHYSICAL SCIENCES

of the

DIVISION OF AIR POLLUTION

- - -

ROBERT A. TAFT

SANITARY ENGINEERING CENTER

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P R O C E E D I N G S

CHAIRMAN SAWICKI: The meeting will come to order. I have a few announcements to make. If you wish a ride down town at 4:30, you have a ride Wednesday and Thursday with the same people who took you yesterday.

Now, we have a recess period of about thirty minutes scheduled for this morning and we are going to shorten it about fifteen minutes because at about 12:30 we have a photographer coming in, and that's it for the announcements.

Now, the first speaker for this session obtained a Bachelors and Masters degree at the University of Kiel in Germany. He obtained his doctorate in 1957 from the University of Munich. He's been working in the field of environmental carcinogenesis at Sloan Kettering Institute with Dr. Wynder. I have seen his laboratory and although he has been working in the field relatively a beginner, I guess all of us are sort of beginners, I was very impressed with his laboratory and I expect to see some very good work coming out of that laboratory. Dr. Hoffmann.

DR. HOFFMANN:

Thank you.

On behalf of Dr. Wynder and myself, I would like to express our appreciation for the opportunity to present our current experimental data in the field of air pollution carcinogenesis before this distinguished audience. We are especially grateful to Dr. Stern and Dr.

1 Sawicki who made this symposium possible.

2 As students of environmental carcinogenesis we
3 have to evaluate first the available human data linking
4 environmental factors to a given type of cancer before
5 interpreting laboratory studies.

6 What is the existing evidence on the relationship
7 of general air pollution and lung cancer? Hammond and Horn
8 in their 44 months follow-up study of 187,783 men showed that
9 there exists a somewhat higher lung cancer ratio in cities
10 compared to rural areas.

11 May I have the first slide,
12 please? These also, compared to this study establish lung
13 cancer cases in groups of known regular smokers and cigarette
14 smokers for different populated areas.

15 This result according to
16 the authors cannot solely be explained by occupational cancer
17 predominantly found in cities. Their study suggests, but
18 does not prove, that general air pollution plays a role in
19 the development of lung cancer. That the urban-rural ratio
20 of lung cancer is not of the same magnitude as the smoker-
21 non-smoker ratio is recently presented by Wynder and Hammond.

22 Please, the next slide.
23 The authors compare in a graph the relative difference of
24 lung cancer mortality over immigrant-native to smoker-non-smoker.
25 In columns 2, 3-A and 3-B, the findings of ^{Eskop} Eskop and ^{Dein} Dein (phonetic).

1 are expressed. These investigators found an excess of up to
2 sixty percent in male immigrants from the United Kingdom to
3 New Zealand respectively South Africa when compared to men
4 born in these countries.

5
6 On the other hand, Hammond
7 and Horn found an excess of more than six thousand for heavy
8 cigarette smokers, forty cigarettes and more expressed on
9 Column 6-C to non-smokers. These data led us to laboratory
10 studies, and the fact remains, according to all investigators,
11 that there exists a somewhat higher rate for lung cancer in
12 city areas compared to rural areas. It is not intended in
13 our laboratory studies to establish proof on the carcinogenicity
14 of air pollutants to men by laboratory studies. Such proof
15 relies today only on human data. But we like to find an
16 understanding and possibly even an explanation for the factors
17 involved in air pollution carcinogenesis which must be in line
18 with the human findings.

19 One approach to this problem
20 is a close cooperation of biologists and chemists. Therefore,
21 I would like to present both our chemical, analytical as well
22 as biological data.

23 The experimental data on
24 the collection system for polynuclear aromatic hydrocarbons
25 and phenols from a V-8 engine have been presented earlier in
this symposium by Charles Begeman from General Motors Research

1 Laboratories, so that I may continue at this point with the
2 report on our analytical data.

3 The dry benzene extract of
4 the engine exhaust condensate and the benzene:methanol extract
5 of the filter system were combined according to the ratio
6 found and a sample of this mixture was used for chemical
7 analysis while the main portion served as test material for
8 the carcinogenicity studies on mouse skin.

9 The first step for the analysis
10 for polynuclear aromatic hydrocarbons was a partition between
11 methanol:water 4:1 and cyclohexane. Thereby we enrich the
12 aromatic hydrocarbons in the cyclohexane phase under separation
13 from the hydrophilic compounds.

14 Please, the next slide.
15 The middle column shows the partition coefficients of various
16 polynuclear aromatic hydrocarbons as phases are used, cyclohexane
17 and methanol:water 4:1. The least favorable coefficient is
18 here listed 14.5, the coefficient for benzo(e)pyrene. In
19 other words, by the distribution between these four solvents
20 we will find more than 93 percent in the cyclohexane area (phonetic
21 so that the three distributions more than 99 percent are
22 extracted for cyclohexane.

23 In order to lose most of
24 the paraffins which as you know would later influence the
25 column chromatography, we enrich the hydrocarbons by further
distribution between nitromethane and cyclohexane.

1 Here shown in the right
2 column was the coefficient from 1.53 for benzo(a)pyrene,
3 3,4-benzpyrene, 60.5 percent are relied upon distribution
4 in the nitromethanella (phonetic), so that five distributions
5 are necessary in order to extract more than ninety-nine
6 percent benzo(e)pyrene with nitromethane and cyclohexane.

7 The dry residue of the
8 nitromethane extracts is chromatographed on neutral alumina
9 (Woelm) of the activity grade II. Hexane, hexane:benzene
10 mixtures and benzene elute the fractions which are taken
11 according to their fluorescence observed on the column with
12 an ultraviolet lamp of the maxima of 365 millimeters. The
13 ultraviolet absorption spectrum of the obtained benzo(a)pyrene
14 main fraction is shown in the next slide. Benzpyrene is clearly
15 indicated by the presence of the peaks 4-1, 384, 362 and the
16 characteristic shoulder at 380, but also indicated is by the
17 shoulder 436 and by benzo(j)fluoranthene 10,11; 31-benzo(b)
18 fluoranthene, 3,4-benzfluoranthene -- no, I am sorry, this
19 is 331, this is benzo(e)pyrene and 301 -- at least this is
20 supposed to be 301. The scale is not on the slide, or the
21 range.

22 Despite repeated column
23 chromatography on silica gel and alumina we did not succeed
24 in obtaining fractions which show identical ultraviolet
25 absorption and fluorescence spectra with those of authentic

reference compounds. For this reason we have chosen paper chromatography on acetylated paper as analytical method for the isolation of pure polynuclear aromatic hydrocarbons. For benzo(a)pyrene, for example, two paper chromatograms were sufficient to lead to benzo(a)pyrene identical with its reference in R_f -value and in ultraviolet absorption and fluorescence spectra. Other hydrocarbons can be obtained equally pure after a single paper chromatogram of the main column fraction, but some require up to five times paper chromatography before definitely showing an entirely identical ultraviolet absorption and fluorescence spectrum to a reference. The latter was the case for dibenzo(a)pyrene, 1,2,3,4-dibenzopyrene.

By repeated paper chromatography we also obtained in one of the late column fractions a reddish-yellow fluorescent band, the extract of which gave a distinct ultraviolet absorption spectrum and also a fluorescence spectrum.

In checking the Wyandotte Punched Cards for ultraviolet absorption spectra of the ASTM, we could not adjoin the spectrum of the isolated compound to any of their recorded spectra up to 1957. In 1958 Aitken and Reid synthesised indeno(1,2,3-cd)pyrene and published its ultraviolet spectrum which we found to be identical with that of our isolated compound.

The next slide, please.

1 This is the isolated compound and this is the R_f -value
2 compound.

3 Yesterday we heard by
4 Badger that one of the pyrolysis products, this component
5 named there ortho phenylene pyrene was also detected in the
6 end of 1959 by Lang and Buffleb also in the pyrolysis product.
7 The fluorescence spectra are also identical with the R_f -value.

8 We consider a polynuclear
9 aromatic hydrocarbon to be present in automobile exhaust gas
10 as well as other combustion products only then when we
11 succeed in obtaining identity with a reference in R_f -value,
12 ultraviolet absorption and fluorescence spectrum or when an
13 otherwise unknown compound does not change its spectra and
14 R_f -value after repeated paper chromatography.

15 The next slide, please?

16 The ultraviolet absorption spectrum of one of the two so far
17 unidentified components from exhaust gas extract is shown here.

18 The ASTM Punched Card sets
19 including the four supplements from 1960 do not suggest that
20 such an ultraviolet absorption spectrum or the fluorescence
21 spectrum did not help identify this component.

22 The infrared spectrum
23 points perhaps to a dibenzene or better a derivative of fluoranthene.

24 In some cases, where larger
25 quantities of polynuclear aromatic hydrocarbons were determined

1 in a sample we were able to crystallize the hydrocarbon from
2 the paper chromatography end fraction after vacuum sublimation.
3 Here, in addition, melting point, mixture melting point and
4 fluorescence of the crystal served as further criteria for
5 the identity with a known hydrocarbon.

6 The next slide, please.

7 In this slide the melting point of five of the isolated
8 hydrocarbons are listed together with the mixed melting point
9 and the comparison of the melting point authentic hydrocarbons.
10 The melting point of the isolated coronene determined in a
11 close melting point was about 4000, however, it did not allow
12 a proper determination of the melting point.

13 The next two slides show
14 the pictures of the crystals of two isolated hydrocarbons --
15 this is the chrysene -- please the next slide -- which is only
16 of academic interest.

17 Next slide, please. This
18 picture was taken at twenty fold enlargement from the microscopic
19 picture whereby 360 millimeter ultraviolet lamp served as
20 light source. This is bens(ghi)fluoranthene. Thank you.

21 The next slide, please.

22 In the next four slides the analytical data summarized which
23 we obtained for gasoline engine exhaust from a "city driving"
24 run at the mileage interval of 8,000 to 12,000 miles. The
25 data in the last three columns represent only the amount of

1 isolated aromatic hydrocarbon as found; in other words, they
2 are not absolute values. Such absolute values can be obtained
3 only with the help of the tracer technique. For this purpose
4 we add a C^{14} -labelled hydrocarbon to the test sample prior
5 to the analytical procedure. We choose the isotopic hydrocarbon
6 in a concentration that we expect according to a pilot analysis.
7 The analysis according to the schedule outlined here leads
8 to a paper chromatography band containing both the C^{14} -labelled
9 and the original hydrocarbon from the test material. The
10 ultraviolet absorption spectrum represents the total isolated
11 amount of the hydrocarbon, and the amount of isolated tracing
12 compound is determined by counting the B-radiation. In this
13 way we are permitted to calculate the absolute amount of the
14 polynuclear aromatic hydrocarbon in the test sample within a
15 maximal deviation of plus minus 5 percent, as shown here for
16 benzo(e)pyrene, 3,4-benzopyrene and benzo(a)pyrene and 1,2-benz-
17 anthracene.

18
19 To our knowledge this is
20 the most accurate method for the determination of traces of
21 polycyclic hydrocarbons in general from combustion materials.
22 It would be rather cumbersome to synthesise all hydrocarbons
23 so far known in combustion products as C^{14} -labelled compounds.
24 Therefore, we try to modify the tracer technique for tritiated
25 hydrocarbons. These tritiated hydrocarbons can be easily
obtained by the Wilsbach Tritiation method and can be counted

1 by the liquid scintillation technique.

2 In our analytical work we
3 have so far mainly worked, however, with benzo(a)pyrene-6-C¹⁴
4 and bens(a)anthracene-9-C¹⁴. Despite equal precautions
5 during the analyses we find a notably higher degree of
6 degradation of the polynuclear aromatic hydrocarbons in an
7 analysis of gasoline exhaust than in an analysis of tobacco
8 smoke products for which we have no final explanation.

9 In column three data are
10 given for the relative activity of the hydrocarbons to mouse
11 epidermis. These results are all based upon data from our
12 own laboratory where the individual hydrocarbons are tested
13 in at least three concentrations on groups of 20 mice each.
14 All hydrocarbons are purified by column chromatography on
15 alumina or "Florisil". Its purity is determined by spectra,
16 R_F-value as well as melting point.

17 Please, the next slide.
18 Our techniques today do not allow the isolation of pure
19 individual alkyl derivatives of polynuclear aromatic hydrocarbons.

20 Ultraviolet and fluorescence
21 spectra together with R_F-value permit to identify individual
22 alkyl derivatives in one fraction, but this fraction will
23 also contain other alkyl derivatives; also when paper
24 chromatography was tried with two different systems. We
25 therefore combined all alkyl derivatives of one hydrocarbon

1 as shown in this slide for benzo(a)pyrene, benzo(a)anthracene
2 and alkyl chrysene. The calculation is permitted since the
3 R_f -value of the alkyl derivatives are not differing above
4 the experimental deviation for the calculation.

5 The last hydrocarbon in this
6 column is benzo(ghi)perylene. Recently we tested this hydrocarbon
7 highly purified cutaneously to mouse skin in .5 and .1
8 concentration. After fifteen months applying this solution
9 we find only one papilloma among 40 mice. In other words,
10 this hydrocarbon was proved to be inactive in our hands.

11 The next slide, please.

12 Indeno(1,2,3-cd)pyrene is weak active according to our latest
13 results, so we should change herefrom a dash to 1 plus. Here
14 the new known equation should be corrected to benzo(ghi)
15 fluoranthene.

16 The next slide, please.

17 Here again we do not determine individual alkyl pyrene or
18 alkyl fluoranthene. We determine only the sum of alkyl pyrene
19 and alkyl fluoranthene, maybe with the assumption that this is
20 all alkyl fluoranthene. Now, here positively are some aryl
21 phenylfluoranthene present, but we were not finally able to
22 identify the individual phenylfluoranthene.

23 In 1959 we reported for
24 the first time the tumor activity of the four benzfluoranthenes:
25 benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene

1 and benz(ghi)fluoranthene. The first two have a moderate
2 tumor activity to mouse epidermis.

3 The next slide, please.

4 In this diagram the carcinogenicity of benzo(b)fluoranthene,
5 3,4-benzfluoranthene and benzo(j)fluoranthene, 10,11-benz-
6 fluoranthene when applied in a .5 percent acetone solution
7 three times weekly to the back of three female mice is
8 expressed in "a month, 10 months," cancer diagram which shows
9 that in several respectively eight and a half months all mice
10 developed skin cancer when this fluoranthene was applied in
11 a half percent concentration to their backs.

12 Several authors, also in
13 the air pollution field, mentioned dibenzo(a,l)pyrene, 1,2,3,
14 4-dibenzopyrene to be a strongly active carcinogen. A thorough
15 study reveals that this compound was tested only once in 1937
16 by Bachmann et al, and it was found that out of two mice one
17 died at the end of six months and the remaining mouse lived
18 for 262 days and developed one epithelioma. In this connection
19 we need to recall that according to the Food Protection
20 Committee of the National Academy of Science a compound is
21 only then regarded as carcinogenic when with the minimum
22 number of three animals a 100 percent tumor incidence is
23 reported. The next figure shows the carcinogenic activity of
24 dibenzo(a,l)pyrene which was synthesized in our laboratory.
25 The carcinogenicity to mouse skin is expressed in the same

1 kind of diagram as for the 2-benzofluoranthene in the last
2 slide with the exception that we tried to extrapolate to a
3 straight line. Using logarithmic probability paper we found
4 the carcinogenic activity for mouse skin of dibenzo(a,l)pyrene
5 between that of benzo(a)pyrene and dibenz(a,h)anthracene.

6
7 At present we are engaged
8 in developing a short term analytical method for the quantitative
9 determination of N-heterocyclic hydrocarbons of which some
10 have been described as carcinogenic in animal tests. We
11 regard a large number of the polynuclear aromatic hydrocarbons
12 together with the N-heterocyclic hydrocarbons as a major
13 group of initiating carcinogens in combustion materials such
14 as automobile exhaust gas. The consideration of initiating,
15 promoting and summation effects as well as competitive action
16 and retardation effects of agents in such complex materials
17 is, of course, a study in itself and we can only step by step
18 aim at an insight into this mechanism. We do know, however,
19 more about some groups of constituents and their role in
20 experimental carcinogenesis.

21 There are the phenolic
22 compounds, many of which accelerate tumor formation as
23 promoting agents to even weak carcinogens.

24 The next slide, please.

25 As an example for the tumor promoting activity of phenols,
this slide shows one of our recent experiments when phenol

1 is applied alone in a 10 percent concentration, only 10
2 percent, two months developed during the testing time. .5
3 percent indene of pyrene gave 20 percent tumor, but both
4 10 percent phenol plus .05 percent indene of pyrene gave
5 during the testing material, testing time of nine months
6 sixty percent tumor. Indene of pyrene alone .05 percent
7 concentration is not active.

8
9 Studies in our laboratory
10 have also shown that phenol and some of its derivatives
11 strongly inhibit cilia movement which underline further its
12 significance in the human environment.

13 Since it is known that in
14 the standard gasoline -- as used for our test run -- a certain
15 percentage consists of aromatic hydrocarbons mainly benzene,
16 toluene and xylenes, it is feasible that these are precursors
17 for phenols formed during the combustion. Therefore, we
18 analysed the automobile exhaust condensate for phenol and
19 phenol derivatives.

20 For this determination we
21 employ a technique developed for the quantitative determination
22 of phenols in tobacco smoke. This method is based primarily
23 upon separation of phenol concentrates by gas liquid
24 chromatography into individual phenols and their quantitative
25 determination by the use of an internal standard. Di-n-octyl
sebacate or polyethylene glycol compound 20M is used as liquid

1 phase; "Gas Chrom P" as support. With helium as carrier gas,
2 best results are achieved at 180°C.

3 The next slide, please.

4 This spectrum represents a typical gas chromatogram as we
5 obtained it from a phenol concentrate of automobile exhaust
6 gas. The phenols are identified by retention time and R_f -value
7 when the fractions are collected and tested on paper. For
8 the quantitative determination of individual phenols we tried
9 our samples before the analysis with one or two phenols which
10 are known to be absent in the test material.

11 The next slide, please.

12 The next slide shows such a gas chromatogram of exhaust phenols
13 concentrate where all the chloro phenols peak Number 1 and
14 peak Number 9, 3,4,5-trimethyl phenol were used as internal
15 samples.

16 The results of the analysis
17 of the benzene extract of a gasoline engine exhaust condensate
18 for phenols is given in the next figure. Here (indicating)
19 this is supposed to be percent. Per benzene extract of the
20 automobile exhaust gas condensate.

21 In brief, the directed
22 analysis of the benzene extract of the gasoline engine exhaust
23 condensate results in 10.5 percent of phenols and approximately
24 2 percent of polynuclear aromatic hydrocarbons of which less
25 than one tenth is known to be carcinogenic to mouse epidermis.

Please, the next slide.

Comparing our results with that of cigarette smoke condensate we would expect a significant higher tumor activity for these automobile exhaust extracts, benzene extracts. Here are the figures. This is the absolute figures determined by the tracer technique. These are only some of the polycyclic hydrocarbons, but the main hydrocarbons which are carcinogenic to mouse epidermis .

The next slide, please.

These are values comparing the phenols and phenol derivatives from cigarette smoke to exhaust gas concentrate.

Therefore, we set up parallel test series with standard cigarette smoke condensate and gasoline exhaust extract. We applied both test materials in acetone solutions of 50, 33, 25, 10 and 5 percent concentration to the shaved backs of 50 female Swiss mice in each group three times weekly for 15 months. Except for the 50 percent concentration the toxicity of both materials indicated by survival rate and weight curve are comparable. Only the 50 percent exhaust extract shows a higher toxicity than tobacco smoke condensate in the same concentration.

The next figure shows a group of four mice treated with 25 percent gasoline exhaust extract for nine months. The second and the fourth mouse from the left show papilloma and the first and third show carcinoma.

1 This kind of papilloma and cancer we observed throughout the
2 whole study. The appearance of papilloma and cancer occurs
3 in general several weeks earlier with gasoline exhaust extracts.

4 The next slide, please.

5 I hope you can see it. This slide shows the first grams
6 recording which we often use in our laboratory for discussion.
7 The green colors indicate the first appearance of papilloma.
8 This, our first is exhaust and should be corrected to automobile
9 exhaust gas, benzene extract, and this should not be tobacco
10 tar, but tobacco smoke condensate, and these are set up in the
11 same concentration; and the red colors identify the first
12 appearance of carcinoma which are all physiologically
13 identified, and the red numbers which you unfortunately can
14 hardly see, are the number of carcinoma. The lower number
15 represents the number of animals that survived. The table shows
16 clearly that the tested exhaust extract is stronger carcinogenic
17 than cigarette smoke condensate.

18 The conversion time from
19 papilloma to carcinoma is with both materials relatively long
20 in comparison to the conversion time observed with pure known
21 carcinogenic aromatic hydrocarbons. With polynuclear hydrocarbons
22 such conversion is in general observed within a few weeks.
23 Comparing the tumor potency according to Blanding the exhaust
24 "tar" has almost twice the potency of that calculated for
25 cigarette smoke condensate. Despite the fact that cigarette

1 smoke condensate contains other known tumorigenic and tumor-
2 promoting agents such as polyphenols and long chain fatty
3 acids the result of this comparison is surprising, when judged
4 on the basis of the chemical analysis. We expected a
5 significant higher activity for the benzene extract of automobile
6 gasoline engine exhaust condensate.

7
8 These results reveal again
9 the difficulty in accounting for the tumorigenic activity of
10 a mixture of such kind.

11 Steiner and Falk as well
12 as Falk and Ketin found some inhibition effects of related
13 carcinogenic hydrocarbons as well as known carcinogenic
14 hydrocarbons, two carcinogenic ones as we heard yesterday by
15 Dr. Falk when such compounds were applied together subcutaneously.
16 This may be one possible explanation for the relative low
17 tumor potency of exhaust extract compared to tobacco smoke
18 condensate. At this time we are studying this phenomenon on
19 mice cutaneously by painting. Early results in our study
20 indicate such inhibition effect for phenanthrene to benzo(a)pyrene;
21 fluoranthene and pyrene rather accelerate the tumor potency
22 of benzo(a)pyrene. These later results, however, need
23 confirmation by repetition of the experiments which are being
24 carried out in various dilutions.

25
Further, we consider it as
important to analyze automobile exhaust gas condensate for

1 long chain aliphatic hydrocarbons since some of them proved
2 to have an inhibition effect on the resorption of carcinogenic
3 compounds. Other aspects which we consider of importance in
4 studying automobile exhaust gas are the operation time of an
5 engine and the different types of fuel. Our results in this
6 regard can be summarized in two tables. The first shows the
7 increase of benzo(a)pyrene as a possible function of the
8 mileage. The benzo(a)pyrene values were determined by the
9 tracer technique and can be, therefore, considered absolute
10 values. This indicates some malfunction of the engine, so
11 I would like to regard this figure not as representative.

12
13 The next slide shows the
14 amount of phenol and benzo(a)pyrene in the exhaust of an
15 engine operated with premium grade gasoline, di-isobutylene,
16 and isooctane.

17 These results indicate
18 again, as many times described in the literature for more
19 than twenty-five years, that almost all organic matter forms
20 to a certain extent polynuclear aromatic hydrocarbons upon
21 pyrolysis. It is also clear from these data that specific
22 precursors for polycyclic hydrocarbons and for phenols reside
23 mainly in the aromatic portion of standard gasoline. Further
24 studies in this direction are intended, including the complete
25 analysis of crankcase blow-by material. In a previous analysis
a considerable amount of polycyclic hydrocarbons was found in

1 the blow-by crankcase oil. Benzo(a)pyrene appeared to be
2 sixteen percent of the total exhaust.

3 Thank you. May I have the
4 lights?

5 In order to determine the
6 extent to which automobile exhaust contributes to general
7 air pollution, detailed analyses of air pollution samples
8 from various city locations and rural areas are underway with
9 the cooperation of the Research Laboratories of the General
10 Motors Corporation. For this study, Mr. Colucci, the paper
11 we heard yesterday is in charge.

12 In summary, the benzene
13 soluble matter of the gasoline engine exhaust condensate of a
14 V-8 engine operated on a "city driving schedule" was analyzed
15 for polynuclear aromatic hydrocarbons and phenols. Twenty-
16 five known and two unknown polynuclear aromatic hydrocarbons
17 were isolated by column and paper chromatography and identified
18 by R_f -values and ultraviolet absorption and fluorescence
19 spectra. Six hydrocarbons were isolated in crystalline form.
20 Nine of the isolated polynuclear aromatic hydrocarbons were
21 confirmed or proved to be carcinogenic in the animal test.
22 Eight phenols were identified in the exhaust gas condensate;
23 five of them are known to be tumor-promoting agents to mouse
24 epidermis.

25 The analyzed material was

1 applied to mouse skin for 50 animals per group in five different
2 concentrations and its tumor activity compared to the
3 corresponding concentration of cigarette smoke condensate. It
4 was found that the tumor potency is about two times higher
5 for the first material compared to the latter. This finding
6 is attempted to be interpreted in terms of chemical constituents.

7 It is emphasized that these
8 laboratory studies merely serve to provide an understanding
9 for the factors involved in air pollution carcinogenesis.
10 In the final instance, they can only be fully interpreted in
11 line with studies involving man. Despite this fact we regard
12 any effort which might lead to a reduction of carcinogens
13 and co-carcinogens in exhaust fumes as most pertinent.

14 We like to express our
15 great appreciation to the Research Staff of the General
16 Motors Corporation who made this study possible. We are
17 especially indebted to Charles Begeman for his close cooperation.

18 This study was accomplished
19 with the excellent assistance of Mrs. Lemm-Hoffmann, Miss
20 Christian, Mr. Theiss and Mr. Rathkamp.

21 Thank you very much for
22 your attention.

23 CHAIRMAN SANICKI: Any questions or comments?

24 Mr. Commins?

25 MR. COMMINS: Commins, Medical Research

1 Council of London. I would like to make some observations on
2 some work we have done in London. I have discovered that
3 in British gasoline we found considerable amounts of polycyclic
4 aromatic hydrocarbons to the extent of up to 2.4 micrograms
5 per millimeter of the gasoline, and for 3,4-benzopyrene up to
6 .4 micrograms per millimeter. Would Dr. Hoffmann -- I have
7 talked to him about this already -- and anybody else like to
8 comment about American gasoline, because I feel this may in
9 some way link the formation of these compounds whether they
10 come from the fuel itself or whether they come from the
11 pyrolysis of smaller molecules in the fuel.

12 DR. HOFFMANN: No, we have not. We have
13 not tested this gasoline originally since we were interested
14 more in the exhaust itself as in the formation since there is
15 the excellent work by Badger that is known and I think we
16 shouldn't go into this field, this is for all the studies
17 that should be done and we will do it and will find that
18 when the premium gasoline we find polynuclear aromatic
19 hydrocarbons, but then we will try with the technique we so
20 far used, and if this is the case, it might be very well.

21 CHAIRMAN SAWICKI: Dr. Falk?

22 DR. FALK: Falk, S. C. California.
23 We did that study because we were interested to see whether
24 our aliphatic hydrocarbons or whatever we got out of our
25 gasoline would have to take into consideration aromatic

1 hydrocarbons that are carcinogenic, and I am talking about one
2 gasoline at one time studied in one careful analysis and I
3 did not see any benzo[a]pyrene whatsoever in our gasoline, but
4 I couldn't tell you for the life of me which gasoline it was
5 and even what year we made the analysis, but it has been done
6 because we wanted to make sure that the gasoline we were
7 using for our purpose then did not contain any of these
8 hydrocarbons.

9 MR. COMBINS: Combins, Medical Research
10 Council. I would like to comment a little further. British
11 gasolines -- I don't know what it's like in America -- we have
12 what we call commercial grades and the higher grades which
13 contain aromatic fractions and in some of the commercial
14 grades we only detected minute traces of these hydrocarbons,
15 but it is in the aromatized fuels where I have detected these
16 hydrocarbons and it is possible that a great deal of the
17 aromatic hydrocarbons detected in the exhaust may come from
18 the fuel itself, although one experiment we have tried with
19 one fuel containing none of these detected hydrocarbons, and
20 another, similar fuel containing some, we have found that we
21 get a reduction of the polycyclic aromatic hydrocarbon content
22 of the exhaust. This is rather peculiar and I don't understand
23 it. It is only one experiment and a third of the concentration
24 of hydrocarbons emitted from the exhaust. The concentration
25 of hydrocarbons in the smoke was the same in both instances,

1 but with the fuel containing the polycyclic aromatic
2 hydrocarbons there was a one-third decrease in total
3 polycyclics.

4 CHAIRMAN SAWICKI: Any other questions or
5 comments? Dr. Katz?

6 DR. KATZ: I would like to ask Dr.
7 Hoffmann whether he found coronene because coronene has been
8 found consistently in our work on air pollution samples,
9 samples of urban air particulates. Another question is this:
10 in connection with your work on the exhaust gases of gasoline
11 engines, did you notice whether there was some correlation
12 between the amount of smoke generated in the exhaust and
13 the concentration of polynuclear aromatic hydrocarbons?

14 DR. HOFFMANN: As summarized -- as we
15 exercised it yesterday with the gasoline work, you have to
16 ask Mr. Eysman because he is running the engine.

17 MR. EYSMAN: We don't see the exhaust
18 gas visually to know whether there is more smoke or not in
19 our experiments.

20 DR. KATZ: I think this is a very
21 important point because one of the ways of controlling
22 polycyclics from exhaust gases is, I believe, to cut down on
23 the production of smoke of all visible exhausts and in air
24 pollution work generally it's been found that polynuclears
25 are correlated as regard to concentration with the smoke

1 fraction of products of combustion. In other words, if you
2 cut down on smoke production then you automatically reduce
3 the polycyclic hydrocarbons. If this can be verified as regards
4 to the automobile exhaust, it's a very important point because
5 otherwise I cannot see how it's possible to hope for a reduction
6 in polynuclear aromatic; if all one can do is simply hope for
7 the products that come out of the engine irrespective of
8 whether you have got an engine that's operated properly or
9 not.

10 DR. HOFFMANN: I think when we would drive
11 our cars with di-isobutylene we would significantly reduce
12 the amount of phenols as well as polynuclear aromatic
13 hydrocarbons in the exhaust, but I think this is a budget
14 question and we cannot afford that; so this is only one
15 academic way, but there are some possibilities; and to the
16 other, we have in mind to further our studies. We have so
17 far, I think, five or six runs and I think that we shouldn't
18 make the five or six runs the final conclusion if there is a
19 relation between smoke, smoke and the amount of polynuclear
20 aromatic hydrocarbons per minute run or per mileage, but I
21 think the figures of Dr. Comins where he finds -- and I
22 think also Dr. Falk -- so I am not absolutely sure -- shows
23 that in diesel engines there is not such a lower amount of
24 polynuclear hydrocarbons present than in gasoline engine exhaust,
25 and I think when you go through cities and see bus driving with

1 diesel engines, there is no doubt about it there is more smoke,
2 exhaust smoke than in gasoline engines, but this is only
3 what I observed. There is no figure, absolute figure. We
4 have only six or seven runs so far and we don't like to make
5 any conclusion from this. Thank you very much.

6 CHAIRMAN SAWICKI: Any other further questions?

7 Dr. Lindsey?

8 DR. LINDSEY: Lindsey, Sir John Cass College,
9 London. I should like to ask Dr. Hoffmann if the cancer
10 producing activity of the fraction which he isolates
11 corresponds quantitatively with the amounts of hydrocarbons,
12 carcinogenic hydrocarbons discovered by quantitative analysis.

13 DR. HOFFMANN: It's too early to make any
14 conclusion because that's not running along, but this is what
15 we have intended only following the lines which Dr. Wynder
16 done was this biological study in cigarette smoke condensate
17 and we like to follow similar patterns.

18 CHAIRMAN SAWICKI: Dr. Falk?

19 DR. FALK: Falk, S.C. We did
20 something of this type that you were alluding to, namely,
21 we had a diesel engine and by the ungrateful cooperation
22 of the Engineering Department -- they hated us for it -- we
23 managed to manipulate this engine either to produce more or
24 less soot just the way we obtained the proper functioning of
25 the engine and we could correlate that the more soot we got, the

1 more polycyclics we got and we actually managed to get this
2 diesel engine to work, so there was absolutely no soot
3 whatsoever coming out under a full load. I don't know what
4 the RPM's were, and we found no benzo[a]pyrene.

5 DR. KATZ: I can corroborate the
6 remarks of Dr. Falk. We have had the opportunity of collecting
7 samples from a 1750 horsepower railway diesel, General Motors
8 Railway Diesel Locomotive. Now, this is a huge engine and
9 it's been set up on an experimental basis at the National
10 Research Council of Canada in Ottawa, Canada, and we have been
11 tied into the project and as a result of that we confirmed the
12 findings of Dr. Cumming and his group that a properly
13 operated diesel will give you far less carcinogenic or polynuclear
14 aromatics than a gasoline engine and in fact going on to what
15 Dr. Falk said, there are times when you get little, if any,
16 aromatics. Most of the material collected is simply -- that
17 is, when the engine is idling -- is simply unburned, the
18 greater part of it is unburned oil, the diesel oil. Some
19 of it comes out unburned, but I think the main difference is
20 the fact that between the two types of engines is that the
21 diesel operates on a high temperature cycle whereas the
22 gasoline engine operates on a comparatively low cycle.

23 CHAIRMAN SANICKI: I would like to bring in
24 one point. Are you trying to say that the amount of
25 particulate is proportional to the amount of benzo[a]pyrene that

1 comes out?

2 DR. KATZ: Well, I wouldn't say
3 benzo[a]pyrene, but --

4 CHAIRMAN SANICKI: Hydrocarbons?

5 DR. KATZ: -- there is a correlation
6 between the smoke or soot coming out of an engine and the
7 total polynuclears. Now, a great deal of this may consist
8 of pyrene and other compounds, so I wouldn't care to relate
9 it to 3,4-benzo[a]pyrene. I haven't seen any published work
10 which is as specific as that, but I think it is related and
11 this ties in with the British work of Cummins and his group
12 that there is a relation between smoke production of a
13 diesel and the polynuclear aromatic fraction.

14 CHAIRMAN SANICKI: Mr. Cummins?

15 MR. CUMMINS: I wouldn't quite agree with
16 Dr. Katz on this point. I think it's a highly complicated
17 study, the relationship between the smoking machine and the
18 polycyclic aromatic hydrocarbon. We have studied recently
19 the pollution in road tunnels under the River Thames in
20 London, and we have found it very difficult indeed to get any
21 correlation between smoke and polycyclic aromatic hydrocarbons.
22 Certainly you can't control diesel engines so they produce
23 very, very tiny concentrations of hydrocarbons, yet vast
24 quantities of smoke. The worst condition I found was following
25 vehicles emitting vast clouds -- these were Lorries -- vast

1 clouds of smoke traveling uphill north of London. There is
2 a hill about a mile long and there we were able to collect
3 smoke from a sampler attached to a private car, and we
4 collected about ten milligrams of smoke, and the concentration
5 of benzo[a]pyrene in that smoke was about no greater than twenty-
6 five micrograms per gram, which is pretty low in comparison
7 with most sources of benzo[a]pyrene and yet we had a lot of smoke,
8 and also when you will maintain diesel vehicles such as
9 London transports, the concentration of benzo[a]pyrene there can
10 be as low as one or less micrograms per gram.

11 CHAIRMAN SAWICKI: Well, we have done a little
12 analysis of the particulate from auto exhaust and we couldn't
13 get any correlation between weight of particulate and amount
14 of the polycyclic hydrocarbon present.

15 Dr. Katz?

16 DR. KATZ: I think this question is
17 tied in with what you mean by smoke. Now, I think I mentioned
18 before that you can get unburned, virtually unburned oily
19 products which do not contain polynuclears, simply mostly
20 composed of the original oil or fuel, however, if you refer
21 to smoke as the incomplete soot product, that's another matter.
22 See, we have got to define our terms.

23 CHAIRMAN SAWICKI: Any other questions? Mr.
24 Begeman?

25 MR. BEGEMAN: Begeman, General Motors.

1 I would like to add a comment to what Dr. Hoffmann remarked
2 in regard to the burning of di-isobutylene, for example, an
3 experiment which we carried out in our engine which Dr. Hoffmann
4 reported on. The di-isobutylene fuel was burned without lead.
5 This is another factor which we think may be significant.
6 The commercial gasoline used had the usual amount of lead,
7 about 300 per gallon, and further studies are being carried
8 out on various leads and we think that might be significant also,
9 and the amount of polynuclear aromatics are emitted.

10 CHAIRMAN SAWICKI: Any further questions?

11 DR. BILLER: My name is Biller from
12 Esso Research and Engineering Company. I think I can add
13 perhaps just a little bit of information to this question of
14 is there any relationship between polynuclear aromatics and
15 the amount of soot as it comes from an automobile engine.
16 I think perhaps we ought to emphasize that there is quite a
17 difference in the mechanism by which you produce smoke or soot
18 between a diesel and an automobile engine. In a diesel it's
19 usually a malfunction; in an automobile engine, even a normally
20 functioning engine can emit soot, and this is dependent further
21 on our fuel ratio. The more fuel you have for the amount of
22 air, the more soot you will get.

23 Now, several years ago we
24 actually did some research in this. It wasn't a very extensive
25 study, so these are rather preliminary results, and we have

1 never recorded them, but what we did find there was that if
2 we ran an engine at different air fuel ratios and these were
3 essentially under no-load conditions, we found that if you
4 compared the total amount of polynuclear aromatics with the
5 organic fraction that was left behind after you extracted
6 with acetone, say this was a very high molecular material,
7 that there seemed to be a fairly good correlation that the
8 ratio of polynuclear aromatics and benzo[a]pyrene to this non-
9 extractable carbonaceous material was fairly or roughly
10 constant; so if you were operating at lean conditions, in
11 other words, we would have expected on that that you wouldn't
12 see very much polynuclear aromatic material in exhaust.

13 Now, the other thing as I
14 remember these studies we found polynuclear aromatics in
15 exhaust from isooctane whether or not it had lead in it,
16 but this again wasn't extensive enough to really point up any
17 differences.

18 CHAIRMAN SAWICKI: Any further questions? Dr.
19 Thomas.

20 DR. THOMAS: Thomas, U.C. Berkeley.
21 This all ties in with the general picture of combustion which
22 you can find scattered throughout the literature, namely, if
23 you start with pyrolysis and then increase the amount of
24 oxygen going through the self-sustaining combustion or auto
25 oxidation region up to stoichiometric conditions and beyond,

1 well, when you come out of the pyrolytic region, you have a
2 large amount of soot produced and a relatively large amount
3 of the condensed polynuclear aromatics and the closer you
4 get to the stoichiometric conditions soot decreases and the
5 condensed polynuclear aromatics also decrease consistent with
6 the point brought out by Dr. Kats, but when you study the
7 overall picture, you have to consider internal combustion
8 where you have to separate as mentioned the auto from the
9 diesel and then you have to consider external type combustion
10 where you are burning in atmospheric pressure as a completely
11 different topic.

12
13 Now, on this railroad, was
14 that operated as diesel or was that a coal burning type engine?

15 MR. COBBINS: Commins. Which engine are
16 you referring to?

17 DR. THOMAS: You mentioned some study
18 done in a tunnel where the railroad operated.

19 MR. COBBINS: No, that was Dr. Kats, I
20 think, mentioned the railroad tunnel. We measured vehicles
21 under road tunnels.

22 DR. THOMAS: Oh, I am sorry.

23 MR. COBBINS: These were motor vehicles,
24 diesel and petroleum vehicles.

25 CHAIRMAN SAWICKY: Dr. Falk?

DR. FALK: I would like to make one

1 more comment that completely ruins everything else that was
2 said. I studied about ten years ago the difference in the
3 output of various polycyclic aromatic hydrocarbons in the
4 exhaust of an engine under different load conditions and we
5 compared pyrene, fluoranthene, 3,4-benzopyrene, 1,2-benzopyrene,
6 coronene and anthracene and benzo[a]pyrene and although we
7 found that there is usually the same pattern if you compare
8 at any time the ratios of all these compounds to each other,
9 they do vary, so there is a condition, a certain amount of
10 lead which will produce more pyrene and less benzo[a]pyrene, and
11 another which may produce less pyrene and more benzo[a]pyrene;
12 so all these correlations are roughly correct, if you increase
13 the lead in the gasoline engine. We decreased all of them,
14 but at lower loads you had different heights at different
15 times and it was a rather confusing situation which luckily
16 nobody is reading because it was published and it did not
17 contribute too much to the understanding of the situation.

18 DR. HOFFMANN: May I make a comment to this?
19 Now, Dr. Falk, we are very well aware of your publication, and
20 I think this was the reason that General Motors Research
21 Laboratories are operating the engine all the time under the
22 same conditions, load and so on, and then I think we have
23 some reason to compare this. Yes, we are very well aware of
24 your publication.
25

CHAIRMAN SAWICKI: Any other questions or comments?

1 DR. ALTSHULLER: Dr. Altschuller, Public
2 Health Service, Cincinnati. I hope we aren't losing sight of
3 the fact that the automobile or diesel is just a test vehicle
4 that we can only operate under certain specific conditions,
5 and you can't control the loading of material going into the
6 atmosphere from millions of automobiles, and furthermore
7 although many of us condense materials, we have got to remember
8 the atmosphere is ventilating at a great rate, and finally
9 many of these compounds of the next speaker who is going to
10 indicate some of the polynuclears are undergoing photo-
11 oxidation reaction in the atmosphere and this is true, I
12 think, on the basis of previous work, and some of the work
13 which we will report next week, for most of the gases of
14 organic species in the atmosphere, they are not stable in
15 the atmosphere, so the relative concentration that one finds
16 when you condense directly from the exhaust may not represent
17 the relative concentration of the material in the atmosphere.
18

19 CHAIRMAN SAWICKI: Now, this is the last
20 comment. We have to give the next speaker a chance.

21 DR. HOFFMANN: Now, may I make a comment
22 on this? We are not having in mind to draw any conclusion to
23 air pollutants or health or lung cancer from this data. We
24 just like to get information about the machines and maybe we
25 can with the experimental biological testing get some
information which is worthwhile to our studies.

1 CHAIRMAN SAWICKI: Thank you, Dr. Hoffmann.

2 Our next speaker is Dr. Kuratsune. He obtained
3 his Ph.D. Kyushu University in 1944. He was at the National
4 Cancer Institute of N.I.H. from 1955 to 1960. He is now in
5 the Department of Hygiene, Professor of Public Health at
6 Kyushu University. He has worked on the determination of
7 benzo[a]pyrene and various pyrogenic materials in oysters and
8 in coffee. Dr. Kuratsune.

9 DR. KURATSUNE: Dr. Sawicki, ladies and
10 gentlemen.

11
12 It is a well-known fact that
13 polycyclic aromatic hydrocarbons are not stable under ultraviolet
14 irradiation particularly in the presence of oxygen. They undergo
15 various chemical changes such as the well-known polymerization
16 of anthracene to dianthracene or the production of photo-oxides.

17 It is rather surprising,
18 however, that very little is known in regard to the magnitude
19 of photochemical decomposition of these hydrocarbons which
20 occur under laboratory illuminations. This is the main reason
21 why the present study was undertaken several years ago.

22 Before the Prinsberg (phonetic)
23 experiment was carried out a preliminary experiment was undertaken
24 in which the effect of irradiation with strong ultraviolet
25 light from a mercury lamp was investigated, since such
irradiation is frequently practiced in the process of cancer

1 analysis of the hydrocarbons. Benz(a)pyrene and 7,12-dimethyl-
2 benz(a)anthracene were chosen for this experiment. They
3 were purified chromatographically and dissolved in purified
4 cyclohexane. The solutions were each taken up into a quartz
5 absorption cell. The cell was made air-tight by placing a
6 thin starch between the cell and its stopper. It was then
7 irradiated by ultraviolet light which was generated by a 100
8 Watt Super High Pressure Mercury lamp and filtered through a
9 Corning filter No. 5970. Thus, the ultraviolet light used
10 for this experiment consisted of mainly 365 and 366 millimicron
11 lines of mercury. Its intensity was 2.2×10^{13} quanta/sec.
12 cm^2 at the surface of the cell as determined by the actinometry
13 with uranyl oxalate.

14 The photochemical change
15 which might occur in solutions during irradiation was
16 pursued by occasional examination of the absorption
17 spectra.

18 May I have the next slide,
19 please? Benz(a)pyrene decreased in concentration by the
20 irradiation while no change occurred when stored in the dark.
21 A similar but more drastic decomposition occurred in 7,12-dimethyl-
22 benz(a)anthracene.

23 May I have the next slide,
24 please? After 130 hours irradiation, all the absorption peaks
25 characteristic for this compound, I mean dimethylbenz(a)anthracene,

1 disappeared completely suggesting the severe destruction of
2 the aromatic structure. This is the absorption spectra before
3 irradiation and this is the absorption spectra after 130
4 hours irradiation.

5
6 Now, I wanted to know
7 whether the photochemical change can be prevented by saturation
8 of the solution with nitrogen. The benzopyrene solution was
9 taken up in a quartz cell and then saturated with nitrogen
10 gas by introducing it into the solution through a fine glass
11 tube. The nitrogen used had been purified by passing it over
12 reduced copper heated at 200°C. The solution was then
13 irradiated.

14 May I have the next slide,
15 please? The decomposition still occurred, but it was much
16 less than when the solution was irradiated in the presence of
17 air. When the solution was saturated with Oxygen and
18 irradiated, the decomposition was most marked, as we expected.

19 In the Princeberg (phonetic)
20 I examined the effect of illumination with fluorescent lamps
21 upon various aromatic hydrocarbons. There is a tendency in
22 modern laboratory illumination that fluorescent lamps replace
23 incandescent lamps. Fluorescent lamps, as you know, emit
24 ultraviolet light, but it seems much weaker in intensity
25 than that generated by mercury lamps. Therefore, it was
examined in the present experiment whether such possible

1 change of aromatic hydrocarbons viewed through fluorescent
2 lamps is negligible or not. Fourteen hydrocarbons were
3 purified and dissolved in purified cyclohexane or dichloromethane.

4 An aliquot, 50 millimeter,
5 of each of the solutions was taken up into 100 millimeter
6 volumetric flasks. The flasks were then sealed with starch.
7 These flasks were uniformly irradiated by 6 40-Watt fluorescent
8 lamps which were located about 160 centimeters over the
9 flasks in a dark room. The lamps were of daylight type most
10 popular in Japan. According to the information supplied by
11 the manufacturer of the lamps -- Now showing the next slide --
12 the lamps generate a relatively small but not negligible
13 amount of energy in wave-length range less than 400 millimicrons.

14 The vertical illumination
15 near the site of the flasks, as measured by an illuminometer
16 was about 1000 Lux and the horizontal illumination was about
17 400 Lux. As control, another aliquot of each solution was
18 taken up in flasks in the same way but was kept in the dark.
19 These flasks were placed in the same room and the temperature
20 in the room was about 30°C.

21 The results obtained were
22 summarized in the next slide. Among the hydrocarbons tested
23 were five compounds including naphthalene, 7,12-dimethylbenz(a)-
24 anthracene, benz(a)pyrene and methyl benz(a)anthracene and
25 anthracene. These compounds decreased in concentration by

1 the irradiation. Naphthacene, as we expected, was most
2 unstable. In Test 1 in which cyclohexane was used as solvent
3 naphthacene decomposed by 80 percent or by about 80 hours'
4 irradiation, 80 percent of the compound decomposed by about
5 80 hours' irradiation.

6 In Test 2 almost 100 percent
7 of the compound present decomposed by 3 days' irradiation.
8 Alkyl derivatives of bens(a)anthracene, for instance, 7-methyl
9 and 7,12-dimethylbens(a)anthracene were also unstable, while
10 bens(a)anthracene did not change under the present experimental
11 condition. The bens(a)anthracene did not change at all.

12 These results agreed with
13 the results obtained by Dr. Cook of England some years ago.

14 Benso(a)pyrene was also
15 labile to the light, decreasing gradually by a prolonged
16 irradiation. Anthracene also seemed to decrease in concentration.
17 The decrease, however, did not progress when the irradiation
18 continued. Right now I am not so sure whether this strange
19 attitude of anthracene is due to some unnoticed experimental
20 error or not.

21 From the control experiment,
22 it was demonstrated that naphthacene and 7,12-dimethylbens(a)-
23 anthracene decreased even when they were kept in the dark.

24 This decrease, however, was much less than when irradiated.

25 The remaining eight compounds,

1 on the other hand, were very stable, showing no appreciable
2 change even by ten days' irradiation. They were stable
3 whether they were dissolved in cyclohexane or in dichloromethane
4 under the present experimental condition.

5 In the next experiment
6 effects of green fluorescent lamps were tested. Theoretically,
7 greenlight does not seem to affect these hydrocarbons. So I
8 once planned to use this type of fluorescent lamp for the
9 illumination of my laboratory. Naphthacene and 7,12-dimethyl-
10 benz(a)anthracene were dissolved in dichloromethane and were
11 exposed to a yellowish-green light generated from six 40-Watt
12 green fluorescent lamps. The spectrum of the light is on the
13 next slide.

14 The light is dominant in
15 green color, but contains a considerable amount of ultraviolet
16 light. The compounds tested decomposed quickly by this light.
17 Nearly all of naphthacene and 60 percent of 7,12-dimethylbenz(a)-
18 anthracene were destroyed by three days' exposure. This is
19 probably due to the fact that in addition to the predominant
20 green light the lamps emit a fairly large amount of energy
21 at the wave-length less than 450 millimicrons which corresponds
22 to the mercury lines. Thus, it was demonstrated that a green
23 fluorescent lamp also is not entirely safe for some aromatic
24 hydrocarbons.

25 In the next experiment,

1 the possible effect of natural illumination, namely, of sunlight
2 which comes in a room through glass windows was examined.
3 Chclohexane solution of 13 hydrocarbons was stored in volumetric
4 flasks and exposed to sunlight in the room. In order to maintain
5 the exposure as uniform as possible, I employed a simple
6 device.

7
8 May I have the next slide,
9 please? The flasks were placed in a circle on a disk in a
10 wooden box. The front part of the box had a window of 3 mm
11 thick plate glass which served as the exclusive entrance of light.
12 The inside of the box was painted black. The disc together
13 with the flasks on it, was slowly rotated by a motor. Thus,
14 the solutions in the flasks were evenly exposed to the sunlight
15 which entered the box through its window. The sunlight coming
16 in from the open air hit first the glass window of the box,
17 then the glass of the flasks, and finally the solutions in the
18 flasks. The light transmission of the glass plate used for
19 the window of the box and of the glass of the flasks is shown
20 in the next slide.

21 The upper card represents
22 light transmission of the glass of the volumetric flasks.
23 The lower represents the transmission of the plate glass which
24 is used for the window of the irradiation box. It is calculated,
25 therefore, that the sunlight which reaches the box will
decrease its intensity by 40 percent at 350 millimicrons and

1 that nearly 100 percent at 310 millimicrons before it reaches
2 the solutions inside. The exposure lasted for five days in
3 the summer, starting at approximately 9:00 o'clock in the morning
4 and ending at 5:00 in the afternoon every day. The intensity
5 of the light entering this box was measured by the illuminometer.
6 A marked hourly, but slight daily variation of the intensity
7 was observed, approximately 6,000 Lux at 9:00 o'clock in the
8 morning, 3,000 Lux at noon, and about 1,000 Lux at 5:00 in the
9 afternoon.

10
11 As shown in the next slide
12 the results obtained were very similar to those of the foregoing
13 experiment. It was noted, however, that the decomposition
14 occurred more quickly than when irradiated with fluorescent
15 lamps. A newly tested compound, 11-benzo(b)fluorene was
16 shown to be very unstable to the light. From this result it
17 may be concluded that prolonged exposure of aromatic hydrocarbons
18 to these artificial or natural light may cause serious
19 destruction of them, although only transient exposure may not.
20 Presence of oxygen affects in a great deal the photochemical
21 degradation of aromatic hydrocarbons. Simple saturation of
22 these solutions with nitrogen, however, may not guarantee a
23 satisfactory protection against the decomposition.

24 Thank you very much.

25 CHAIRMAN SANICKI: Any questions or comments?

Dr. Hoffmann?

1 DR. HOFFMANN: Dietrich Hoffmann, Sloan
2 Kettering Institute, New York. Original study by Wynder and
3 Wright were done with dichloromethane as elutes (phonetic)
4 in solvents and later it was found that this solvent also when
5 kept in the dark is reason for some changes, quantitative
6 changes in benzo(a)pyrene and other polynuclear aromatic
7 hydrocarbons despite the fact that it was highly purified;
8 and the other point we observed also was the photo-oxidation
9 especially when preparing for animal experimentation polynuclear
10 aromatic hydrocarbons we came to the point that we have to
11 purify every two weeks our polynuclear aromatic hydrocarbons
12 to be assured that our experiments can be repeated.

13 In the analytical group
14 we cover our lamps with yellow plastics named butyrate tenite
15 (phonetic) which absorbs all light below 470 millimeters.
16 Then we exposed solutions in this room to the light and
17 observed this cyclohexane as solvent and observed this
18 ultraviolet absorption spectra and we couldn't find any more
19 emission.

20 CHAIRMAN SAWICKI: Any other questions?

21 Mr. Commins?

22 MR. COMMINS: Commins, Medical Research
23 Council. I am very interested to hear of your results. We
24 have studied the effect of sunlight on solutions of 3,4-benzopyrene.
25 I would like to ask you what amounts of hydrocarbons you had

1 in your solutions because we found that strong solutions of
2 benspyrene, as an example, remain reasonably stable. They go
3 slightly yellow, but the concentration remains more or less
4 constant. A weak solution goes very rapidly indeed, and I
5 would emphasize this point people making up standards for spectro-
6 photometry analysis should make up the standards immediately
7 because the effect of sunlight is quite marked on the solutions.
8 Just the light in the laboratory is enough in weak solutions
9 of pure hydrocarbons, for extracts they are much more stable.

10 DR. KURATSUNE: I used benspyrene solution,
11 about five gram per millimeter.

12 CHAIRMAN SAWICKI: Dr. Lyons?

13 DR. LYONS: Lyons, New York. I think
14 the reason why weak solutions degrade more quickly is due to
15 the fact that in the strong solutions the first few layers of
16 molecules absorb most of the instant energy, so when you were
17 irradiating your solutions, I think it would have been better
18 if you agitated the solutions.

19 DR. KURATSUNE: No, I didn't.

20 DR. LYONS: Did you agitate them?

21 DR. KURATSUNE: No, I didn't agitate them.

22 DR. LYONS: I think a lot of people do
23 that.

24 DR. KURATSUNE: Yes, I know that.

25 CHAIRMAN SAWICKI: I think another factor there

1 would be the relative proportion between the oxygen solution
2 and the hydrocarbons, of course.

3 Any other questions? Dr.
4 Altshuller?

5 DR. ALTSHULLER: In the atmosphere itself
6 would this photo-oxidation be of any importance? In other
7 words, I wonder would there be enough of the polynuclear
8 material on the actual surface of the particle to be of any
9 importance, because here, of course, you wouldn't have the
10 added protection of your window glass etc., you would have the
11 complete solar radiation and also, of course, in oxygen type
12 smog such as Los Angeles you have a secondary reaction of
13 possibly of the polynuclears with the ozone atomic oxygen
14 produced from the pyrolysis of NO_2 . Would you please comment
15 on that?

16 CHAIRMAN SAWICKI: Mr. Commins, would you
17 like to comment on that? You have done a little work in that
18 line.

19 MR. COMMINS: I really don't know an
20 awful lot about ~~oxides~~ of nitrogen, but NO_2 itself certainly
21 does decompose benzpyrene; if you coat filter paper with
22 benzpyrene ^(and pass it through?) possibly nitrogen dioxide it goes yellow immediately,
23 but I perhaps would like to leave my comments until tomorrow
24 when I will talk about the relative stability of samples in
25 some detail, if that's satisfactory to you.

1 CHAIRMAN SAWICKI: Sure. Dr. Falk?

2 DR. FALK: Falk. U.C. I tried to

3 answer some of your points yesterday when I showed the slide
4 where the exposure of the aromatics to smog and to light showed
5 disappearance, but in this study we did not differentiate
6 between oxidated disappearance and just sublimated disappearance.
7 The compounds that do not seem to be affected as shown by
8 Masanori Kuratsune which disappeared happily in my study were
9 all the smaller compounds which obviously seem to sublime easily
10 while the ones that also sublime but also seem to disappear
11 out of proportion to the others might have been oxidised, and
12 the smog sure helps. We can make nitrobenzopyrene if we so
13 desire. It has a different spectrum.

14 CHAIRMAN SAWICKI: Any other questions or
15 comments? Thank you, Dr. Kuratsune.

16 We will have a recess
17 at this time till 10:45. Thank you.

18 (Short recess.)

19 CHAIRMAN SAWICKI: The meeting will come to
20 order. The first speaker for this part of the meeting I am
21 not going to introduce because I have introduced him amply.
22 I think it will go to his head if I introduce him again, so
23 Dr. Thomas will talk on the mechanism of fluorescent spectroscopy.
24 Dr. Thomas.

25 DR. THOMAS: Dr. Sawicki.

1 The use of fluorescent
2 spectrophotometry as a tool to measure and identify the
3 aromatic fluorescent compounds which can be found in polluted
4 atmospheres is very limited because there are an infinite
5 number of spectra which can be obtained from a single solution
6 depending primarily on the optics and the geometry of the
7 measuring system.

8 May I have the first slide?

9 Now, this is a schematic representation of a particular
10 fluorescent spectrophotometer, but the arrangement is quite
11 standard for all spectrophotometers. You have a light source,
12 in this case it's a xenon lamp. This has become quite standard,
13 and to get the incident light of the desired wave length that
14 passes through the first monochromator into the sample housing,
15 the hatched area showing the position of the fluorescent sample,
16 top view of the cuvette, the entrance slit and exit slit are
17 indicated generally serving to collimate the incident light
18 and the fluorescent light. The fluorescent light is then
19 passed into a second monochromator. In this particular
20 case I have indicated a Beckman spectrophotometer and the
21 fluorescent spectrum then is picked up by a recording device
22 from the signal sent into the photo tube housing.

23 The instruments that are
24 on the market, the Aminco instruments and the Fahren have
25 their arrangement in a similar fashion. Of course, the

1 monochrometers in the one case being the Aminco monochrometer
2 and in the other case being the Fahren monochrometer, but
3 the physical arrangement is identical.

4 We had to use this make-shift
5 arrangement, and I say it's make-shift, because it's probably
6 the poorest arrangement because we wanted to vary the position
7 of the entrance and exit slits which is not possible with any
8 of the existing spectrophotometers that are on the market.

9 May I have the next slide,
10 please? The upper left-hand portion shows the cuvette containing
11 the fluorescent material indicating the entrance slit and the
12 exit slit. Moving over to the top right we have the relative
13 positions of the entrance and exit slits indicated and the
14 fluorescent spectrum of a single solution are indicated below.
15 This happens to be for Benzo(a)pyrene, and you will notice
16 that there are four different spectra for the single solution.
17 Not only does the intensity vary, but you will notice the
18 relative intensity of adjacent peaks can be varied just by the
19 changing of the position of the entrance and exit slits.

20 Here we have the peak at
21 403 and at 426. 403 is higher than 426 and in this case we
22 have the reversal where the 426 peak is larger than the 403
23 peak, so this will give you an idea of the large number of
24 curves that can be obtained from a single solution varying
25 the position and the width of the entrance and exit slits.

On the basis of this simple experiment it was possible to derive an expression for fluorescent spectrophotometry relating the intensity of fluorescent light at one wave length to the intensity of the incident light at another wave length and the relationship is comparable to the Beer Lambert Law for transmission or absorption spectrophotometry.

May I have the next slide, please? This is an enlarged top view of the sample cuvette. You will notice the top there is an "L". This "L" generally is equal to one centimeter inside dimension for the cuvette commonly used on spectrophotometers. I am not interested in the entire cuvette as indicated. I am only interested in the small segment within the cuvette.

The incident light passes through the entrance slit as indicated on the right-hand side, and the intensity of fluorescent emission is measured at right angles to the incident light and the transmitted light. The fluorescent light passing out through the exit slits. The co-ordinate positions of the entrance and exit slits are indicated by "Y-1" and "Y-2" and "X-1" and "X-2" accordingly.

The absorbed light in increment "DX" may be obtained by means of the Beer Lambert Law where we consider the transmitted light through "X" centimeters of solution and the transmitted light through "X"

1 plus "DX" centimeters of solution.

2 May I have the next slide?

3 This is merely a review of the Beer Lambert Law. Now, instead
4 of "L" which in conventional spectrophotometry would correspond
5 to the entire width of the cell, if I am interested in the
6 transmitted light at "X", I substitute "X" for the "L", and
7 if I am interested in the transmitted light at the point "X"
8 plus "DX", I substitute "X" plus "DX" for "L" and the symbols,
9 I think, are quite conventional.

10 May I have the next slide,
11 please? Therefore, to obtain the light absorbed in increment
12 "DX", "DY", I consider the light transmitted through "X" and
13 subtract the light transmitted through "X" plus "DX", you
14 can see I have made the substitutions here expanding, simplifying
15 and applying the limits as defined by the exit slit, I come
16 up with an expression to obtain the light absorbed in increment
17 "DX", "DY" as indicated. In Equation 1, now I will use Equation
18 1 later on. I hate to ask you to remember it because there
19 are a number of these expressions, but I will use Equation 1
20 later.

21 May I have the next slide,

22 please? Now, the compounds fluorescent compounds, if they
23 absorb a portion of the -- or a portion of the incident light
24 that is absorbed by fluorescent compound may go to form the
25 fluorescent light, either a part or all of the incident light.

Now, the expression used to measure this conversion or the conversion factor is defined as "P" and it is called the fluorescent efficiency or quantum yield. In the expression which we have developed, we have used "P" in a similar manner defining "P" not as the quantum efficiency relating the total absorbed energy to the total emitted fluorescent energy, but rather we have signified "P" lambda where this is the wave length of interest as the relationship between the intensity of fluorescent light at one particular wave length to the total absorbed energy. From Equation 1 we have the absorbed energy in the increment "DX", "DY"; multiplying by the conversion factor "P" we now have the fluorescent light which is contained within the solution.

Now, as indicated earlier there was a "DY" that was carried all the way along which is the entrance slit and this is finite and applying the limits "Y-1" and "Y-2" to "Y", we can define the fluorescent light in the contained within the solution in the increment indicated on one of the earlier slides in the top view.

May I have the next slide, please. Now, applying Beer's Law to the fluorescent light contained within the solution, we can come up with an expression for the intensity of the fluorescent light that is emitted from the solution. Applying or substituting

Equation 1 and Equation 2 into this expression and applying the appropriate limits for the entrance slit, we come up with this expression which when integrated out we come up now with the expression for fluorescent spectrophotometry, and this is the expression I mentioned which is comparable to the Beer Lambert Law. Actually it is not too much different even though it looks a little more hairy the symbols to indicate the incident light at one wave length and the fluorescent at another wave length and we are concerned with "P" at another wave length and we have absorption of light not only of the incident light, but we have absorption of the fluorescent light, so we have the "K-1" and the K-0". These are the same "K's" that are used in the Beer Lambert Law and "C" corresponds to the concentration expressed as the Moeler concentration comparable to the Beer Lambert Law.

The distances are measured in centimeters again comparable to the Beer Lambert Law.

To be universal the expression must include height. We arbitrarily selected one centimeter.

Now, the height is determined by the effective height of the photo tube and one centimeter is well within the effective height of most photo tubes. Therefore, the fluorescent light contained within the solution is contained in a rectangular prism defined by this arbitrary height, the entrance and the exit slits. If maintaining the same slit program in the same

1 program throughout the fluorescent measuring system, we
2 substitute a plainer mirror bisecting diagonally the prism
3 containing fluorescent light, we can measure the incident light
4 in the same arbitrary units as we measure the fluorescent
5 emitted light. Everything else being constant, we can calculate
6 out absolute values for "P" at a particular wave length for
7 a particular compound. You must realize that corrections
8 must be made for the characteristics of the light at each
9 wave length.

10
11 May I have the next slide,
12 please? Now, at low concentrations it is possible to effect
13 a simplification of the general expression and expanding,
14 simplifying, we come up with the expression whereby the
15 intensity of the fluorescent light is proportional to the
16 concentration. This is consistent with empirical observations
17 widely used throughout fluorescent spectrophotometry. Those
18 that are interested in doing quantitative work realize that
19 you can get a straight line plot at very low concentrations
20 of the fluorescent intensity with the concentration, and the
21 reason behind it can be determined in this simplification
22 although there is another more simple explanation, but basically
23 it comes back down to the same thing as presented on this slide.

24 The next four slides will
25 indicate the validity of the fluorescent expression.

May I have the next slide,

1 please? We have a comparison of the calculated and observed
2 values of the fluorescent intensity of perylene at various
3 concentrations, the observed being the solid and the calculated
4 the dotted where we have now the intensity of fluorescent
5 on the left side and the concentration on the right-hand side.
6 This is expressed in "C" value, "C-7, C-6, -5" and so on
7 where "C-0" is slightly less than the saturated concentration
8 and then there is a serial solution where "C-1" is one half
9 the concentration of "C-0", "C-2" is half the concentration
10 of "C-1" and on down so that "C-11" is half the concentration
11 of "C-10".

12 The Beer Lambert region,
13 in other words, the region in which we can reliably accept
14 values for "K", extends out to about seven in this particular
15 case, and you will notice that there is a fairly good
16 correlation between the calculated and observed, and the
17 lower concentrations, as the concentration increases, there
18 begins to appear a larger deviation, but this is reasonable
19 as we use the same values of "K" extending beyond the Beer
20 Lambert Law region whereas there are unique values of "K"
21 out at these concentrations and if we use these unique values
22 of "K", we have tried it in a couple of instances, we can bring
23 these points right back up so that the calculated and observed
24 fall on top of one another.

25 Now, these correspond to

¹ the peaks at 438 millimicrons, 465 and 499.

The region that is conventionally used for quantitative determination is down oh, "C-10", maybe pushing out to "C-9". There is a linear relationship at this point and orders of magnitude wise this corresponds to micrograms of material per CC of solution, but you see that you can extend beyond the linear range out into the range where you start to bend over. Some of the other curves have a greater bend than is indicated here.

Now, the slope is determined by "P" values and "K" values and this will indicate why some compounds, for example, benzo(a)pyrene, I have a curve for benzo(a)pyrene, why it is much more sensitive. You can get down to much lower concentrations as you have a different slope than indicated on this curve for perylene.

Now, the expression may be used to -- I don't know whether I want this particular curve or the next one. Can I try the next curve to see if it has the point on it that I am interested in? I might have to come back to this curve. Can I go back to the previous curve? Is it possible? I have to go to actually two curves later on. We will quickly look at this next curve as we pass through it and I will explain it. This is the curve for anthracene. Again a different concentration, different wave length involved. You will see that there is a good correlation between calculated

1 and observed. Again there is a deviation when you get out
2 beyond the Beer Lambert Law region. The slit in the first
3 slide and in this slide, there are face, center, entrance
4 and exit slits. The slit width being in this case five tenths
5 of a millimeter or .05 centimeters.

6 May I have the next slide,
7 please? Here we have a comparison between the calculated
8 and observed for benzo(a)pyrene. We have the 403 curve, the
9 426 and the 452. Again the same comments that were previously
10 made hold for this particular slide. The entrance slit in this
11 particular case was open from .04 to .05 centimeters.

12 May I have the next slide,
13 please? This will bring out the point that I wanted to make.
14 It's possible by means of this -- of the expression to explain
15 the phenomena whereby increasing the concentration you get an
16 apparent change in the fluorescent, in the color of fluorescence.
17 Now, this occasionally occurs down in the Beer's Law region
18 as indicated on this slide. We have the intensity of the
19 peak at 435 which predominates up to a concentration corresponding
20 to C-6 which is in the Beer's Law region; then as we go beyond the
21 concentration corresponding to C-6, now the curve at 465 begins
22 to predominate and we get the color change that is sometimes
23 observed as you increase the concentration for the fluorescence.
24 Now the reason behind that, you have a very characteristic
25 fluorescent spectrum. There are a number of peaks and with

1 each peak there is a "P" and an absorption coefficient
2 associated, and when you stick in the appropriate values of
3 "P" and calculate out "IF", you can come out with the values
4 indicated. Now, if I may, if possible, go back to the
5 previous slide, the one showing benzopyrene. Now, this
6 expression on this slide and using the proposed expression
7 for fluorescent spectrophotometry explains the phenomenon
8 that is commonly called concentration quenching. In other
9 words, as we increase the concentration the fluorescent
10 intensity reaches some maximum and then falls off. Possibly
11 I am not going to comment on quenching per se because the
12 subject is very complex and I am in this particular instance
13 interested in the mechanism of a spectrophotometer, but rather
14 than concentration quenching, I think a better expression
15 would be fluorescent absorption or self-absorption because
16 it is really an absorption phenomenon which is responsible
17 for the breaking of the curves which I think has been observed by
18 all of us that are playing around with fluorescence.

19
20 Now, the term self-absorption
21 has been used throughout the literature, so there is nothing
22 new in that expression, and I think it probably is more
23 meaningful than the expression concentration quenching. I
24 have shown a graphical comparison. I also have the figures
25 with all the information on additional slides, but I think
it's pointless to throw all this numerical data at you. We

1 will just leave it go at this point. Thank you.

2 CHAIRMAN SAWICKI: Any questions or comments?

3 MR. BEGEMAN: Mr. Begeman, General

4 Motors. I missed the comment if you made one on the solvent
5 you use in your system.

6 DR. THOMAS: We used cyclohexane throughout.

7 The solvent doesn't enter into the expression, but there
8 would probably be different values of "P" and, of course, "K".
9 The comparison is identical between the conventional treatment
10 as we have used Beer's Law in this particular expression.

11 CHAIRMAN SAWICKI: Any other questions? Dr.
12 Falk?

13 DR. FALK: Falk. S.C. Would you like
14 to comment on the picture that you showed in the first slide
15 where you had not changed concentration, but where you got
16 different maxima, different heights of the maxima just on
17 the basis of geometric changes in your slide? Do you remember
18 the four different --

19 DR. THOMAS: Maybe we could go back to
20 that first slide. It would be the second slide, I believe,
21 in the series.

22 DR. FALK: Yes, this one here. In A,
23 B, C, and D.

24 DR. THOMAS: The slide or position
25 corresponding or as indicated in "A", we have most of the

1 incident light is absorbed before it reaches the point out
2 here (indicating). A small amount of light that is fluorescent
3 light within the solution is further absorbed as it passes
4 out explaining the very small curve that we obtained as
5 indicated under "A".

6
7 Under "B", the incident
8 light does not pass through a large value of "X", so we have
9 a large amount of fluorescent light at this particular point
10 and the fluorescent light then passes out and there is a
11 certain amount of absorption that occurs of that fluorescent
12 spectrum, and we have the curve as indicated at "B".

13 Now, "C", this would be,
14 if you wanted to get the greatest sensitivity out of a
15 spectrophotometer design, you would arrange your slits in
16 this position where the incident light passes through the smallest
17 amount of solution and the fluorescent light contained within
18 then passes out through the smallest amount of solution. We
19 have a curve corresponding to "C" where this is the highest
20 curve, and you will notice here we have a reversal. Now,
21 using the values for "P" and "K", "K-1" at this point and "P"
22 and "K-1" at this point, the calculations show the reversal
23 as indicated in your developed expression. Then we have
24 Expression "D" where most of the incident light is absorbed
25 and we have a small amount of fluorescence, but it has to pass
out through just a small amount of solution, so we have this

1 curve which is a little higher, curve "D" higher than curve "A".

2 DR. FALK: When you came to the picture
3 that you showed us about benzopyrene versus concentration
4 ranges, you did not have that overlap that you have over
5 here. Remember the other slide later on?

6 DR. THOMAS: That was for a single
7 program on the spectrophotometer. I wouldn't have --

8 DR. FALK: You had all the three
9 maxima there next to each other floating around.

10 DR. THOMAS: Yes, but you see, I kept
11 the fixed slit arrangement. In this particular case there
12 are four different programs on the curve that I compared.
13 There was one program for the benzopyrene. It was a face
14 centered slit. That work, incidentally, was done at the
15 California State Department of Public Health for the use of
16 their Aminco Bowman spectrophotometer. That was obtained on
17 an Aminco Bowman spectrophotometer. The anthracene curve was
18 on the Teer's phosphorimeter (phonetic) which I think is what
19 you have in your lab. Again you are stuck with the face
20 centered slit arrangement and the slits, I think, your slits
21 are measured in the Teer's in millimeters where their's is
22 in -- still indicated in inches and I used the smallest possible
23 slit arrangement so I could get the sensitivity so I could get
24 enough resolution in the curves and the slit, I think, was .05
25 centimeters. Actually that's the lowest you can get, .5

1 millimeters. One of the curves that was shown, the final
2 curve, was one where I used the make-shift spectrophotometer
3 which I indicated on the first slide that I showed where I
4 had the entrance slit was the entire face of the cell and the
5 exit slit was two tenths of a millimeter off center.

6 Now, we have tried other
7 arrangements and the equation works out as well as indicated
8 on this schematic drawing.

9 CHAIRMAN SAWICKI: Any other questions?

10 DR. TIPSON: Tipson, Bureau of Standards
11 in Washington. When you press the button and let the light into
12 the instrument and take a fluorescence reading immediately
13 then repeat again and again, do you always get the same reading
14 or do you get a diminishing every time you press the button
15 and take the reading?

16 DR. THOMAS: Well, some compounds are
17 more sensitive to light than others. In this particular case
18 we tried to take compounds and concentrations. We assumed
19 that there was no change with respect to concentrations even
20 though we knew that there was some change occurring. These
21 compounds were slightly affected by the light. Now, this is
22 a slow process. There isn't an instrument on the market that
23 would give us the readings that we were after. The Aminco
24 does give you the scan of the fluorescent spectrum. We could
25 scan very rapidly, but we were interested in a point where we

1 would check our points at different wave lengths, so it was all
2 manual going back to the original work that had to be done
3 on the spectrophotometer where we manually plotted out all
4 of these points because there isn't an instrument on the
5 market that would give you absolute readings as what we were
6 after.

7 CHAIRMAN SAWICKI: Any other questions or
8 comments? Thank you, Dr. Thomas.

9 Our next speaker obtained his Doctor of Science
10 at the University of South Africa in 1951; did post doctoral
11 research with Dr. Roger Adams on the structure monocrotaline.
12 He also did post doctoral research with Dr. Cram on the synthesis
13 of sperononaene. He did quite a bit of work on column and
14 paper chromatography, uv and visible absorption and fluorescent
15 spectroscopy. He has identified quite a few polynuclear
16 aromatic hydrocarbons in cigarette smoke condensate and I
17 believe he's done a very nice job in this, and here's Dr. Van
18 Duuren.

19 DR. VAN DUUREN: Thank you, Dr. Sawicki.
20 Although the chemical work in our laboratory has never been
21 directly in the field of air pollutants, we have carried out
22 some experiments in arial environmental carcinogens that may
23 be of interest to this meeting.

24 Our work on the chemistry
25 of cigarette smoke is, of course, now all in the literature

1 and we have been active in other areas for the past three
2 years. However, guided by questions I have received from
3 visitors to our laboratories, I would like to make a few brief
4 comments concerning methods of separation and identification.

5 Column chromatography did
6 not in our hands ever give fractions that were clear enough
7 for identification by UV as shown in the first slide. This
8 shows the UV of a column chromatographic fraction, UV of a
9 column chromatographic fraction before and after paper
10 chromatography. This is after one paper chromatogram. You
11 can see that the column chromatographic fraction shows
12 just the barest signs of peaks of benzopyrene.

13 It should be noted though
14 that cigarette smoke is a very complex mixture of many
15 hundreds of organic compounds and column chromatographic
16 procedures are probably and very likely more effective with
17 less complex mixtures such as air pollutants.

18 We use then paper
19 chromatography followed by UV and fluorescence spectroscopy
20 for the identification of these compounds. Some typical
21 paper chromatograms are shown in the next slide.

22 This again points out the
23 difficulties in trying to separate aromatic hydrocarbons
24 directly by column chromatography. These are all cigarette
25 smoke fractions that were obtained after extensive column

chromatography and then were separated by these paper chromatographic procedures.

Where we wanted to separate larger quantities of materials, material was applied in a banded separation shown here. All these separations were obtained with Tarbell's procedure using dimethylformamide and hexane with Whatman number 1 paper. Others workers have used with equal success other methods, for example, the Bergman's toluene methanol water system and Wieland's system with methanol, ether and water. Still other methods are available for use.

It is often advantageous, however, to use more than one, two or three paper chromatographic systems in succession as shown in the next slide.

These dibenzscarbazoles which we have looked for in cigarette smoke and some of which we found you can see cannot be separated at all on Whatman number 1 paper, their R_f 's are the same. On acetate paper they can be separated. The dibenzacridines also we had examined by these two procedures. Using these two systems successively we were able to separate the dibenzscarbazoles and dibenzacridines from cigarette smoke condensate.

The question is often asked me which paper chromatographic system I would consider

1 to be the most advantageous. Unfortunately I cannot answer
2 that question. We were interested primarily in the
3 identification of aromatic hydrocarbons and heterocyclics,
4 particularly the carcinogenic ones, in cigarette tars and
5 once we found a few systems which worked we did not examine
6 the potentialities of any of the systems or their relative
7 merits or drawbacks.

8 We have found, however,
9 and have looked in the question of recoveries from paper
10 chromatograms. We have found that we can get with the
11 dimethylformamide and hexane system recovery of 95 percent or
12 better from spots on which one microgram or lesser material
13 was chromatographed. With larger quantities of material
14 considerable losses were entailed due to trailing.

15 We have established with
16 our paper chromatographic systems and the hydrocarbons with
17 which we deal the quantities of material which give the best
18 recovery values.

19 The chemical and analytical
20 procedures which we had used in the cigarette smoke work were
21 extended more recently by us in another subject and that
22 concerns the study of the metabolism of aromatic carcinogens.
23 Since these metabolic products are all derived by oxidation
24 of the carcinogen they may be of concern to those in air
25 pollution research who are interested in finding and analyzing

1 such oxidation compounds of aromatic hydrocarbons.

2 Dr. Kuratsune has discussed
3 already this morning the question of photo oxidation and in
4 that sense I felt you may be interested in a few of these
5 results.

6 The metabolites which we
7 studied were obtained from the rat lung metabolism of
8 dibenz(a,h)anthracene. The products were all obtained in
9 microgram and submicrogram levels and their identification
10 presented to us a new challenge. Prominent among these
11 products was a 5,6 and 7,14 quinones of dibenz(a,h)anthracene
12 shown in the next slide.

13 Now, these are the two
14 quinones. They do not ordinarily fluoresce in organic
15 solvents and remain at the origin of paper chromatograms
16 with most of the solvent systems we used. The UV adsorption
17 spectra show broad bands which are not readily distinguished
18 from background adsorption. We use the well known chemical
19 reaction, reductive acetylation with zinc dust and acetic
20 anhydride to convert the mixture of quinones from the
21 metabolism of D.B.A. to the respective hydroxy-acetates as
22 shown.

23 Also, the ortho-quinone
24 forms a strongly fluorescent quinoxaline with ortho-
25 phenylenediamine, and all three of these compounds could be

1 readily separated, paper chromatographed and their
2 fluorescence spectra obtained.

3 In the next slide we have
4 fluorecence of the 3,4-diacetate showing the metabolic
5 product and the authentic compound, and in the next slide the
6 7,14-diacetate.

7 Now, another metabolite
8 which we found from dibenzanthracene was dihydrodiol shown
9 in the next slide. This compound gave a very nice spectra
10 which compared quite well with that of the authentic compound
11 but there was some question about its identification because
12 it has the phenylphenanthrene moiety and several other
13 compounds with the same phenylphenanthrene moiety
14 and without hydroxal would show very similar both ultra
15 violet and fluorescence spectra.

16 So that we needed in that
17 case some additional confirmation. The presence of the
18 compound was confirmed by the reaction sequence which we have
19 shown in the next slide. In other words, we treat the
20 compound with acid, lose its molecule of water to give the
21 corresponding phenol which is treated with diazomethane which
22 converts it to the ether. These ethers are very readily
23 paper chromatographed using the same solvent systems that
24 we used for the hydrocarbon itself and are, of course, are
25 found fluorescent so that we could measure this compound and

1 determine very definitely its presence.

2 That is all that I had
3 planned to say on metabolism work since that is not of major
4 concern to the audience here. I would like to turn now to
5 another aspect and that is one which has already been
6 discussed this morning, the question of fluorescence.

7 The use of fluorescence
8 as an analytical tool in identification of aromatic
9 hydrocarbons in heterocyclics has, of course, expanded
10 tremendously and the past few years. Some exploratory work
11 along these lines were carried out also in our own laboratory
12 and some aspects of this work I think I can appropriately
13 discuss here.

14 We studied the effects
15 of solvents on the fluorescence of a group of heterocyclic
16 compounds. While these compounds are not likely air
17 pollutants or for that matter even carcinogens these results
18 are of interest since they point to the potentialities of
19 the examination of such solvent effects for analytical and
20 assay purposes as was already pointed out yesterday by Dr.
21 Falk.

22 There is, in fact, already
23 some work along these lines in the literature. One such case
24 is shown in the next slide. This shows the UV adsorption
25 spectrum of 1,2,5,6-dibenzacridine in cyclohexane and in

1 sulfuric acid. Remarkable shift to longer wave length which
2 is also reflected in the fluorescence spectra shown in the
3 next slide. Again we notice the shift to longer wave length
4 in both the excitation and emission spectra.

5 Now, this is typical of
6 the usual solvent effects that have been examined and
7 described by previous workers. In compounds, however,
8 there are shifts in the fluorescence emission spectra that
9 are not accompanied by comparable shifts in the UV adsorption
10 or fluorescence excitation spectra.

11 We have examined a few
12 compounds of this type in fair detail. Let us look at the
13 UV adsorption of indole shown in the next slide. This shows
14 the UV adsorption of indole in a series of solvents,
15 cyclohexane, water, dioxane, ethanol and a dilute aqueous of
16 sulfuric acid. It is clear that the change of solvent has
17 little effect on the band positions so that the resolution
18 and to a minor extent intensities are affected.

19 The fluorescence excitation
20 maxima of indole in these various solvents were also identical.
21 However, large changes in the fluorescence emission are
22 brought about with change of solvent and some of these are
23 shown in the next slide. All these spectra were obtained
24 at the same concentration of five and a half micrograms per
25 millileter and at the same excitation wave length 282

1 millimicrons.

2 This diagram shows the
3 emission of indole in cyclohexane, dioxane, water and ten
4 percent aqueous sulfuric acid, the shift of 53 millimicrons
5 in going from cyclohexane to water is noteworthy.

6 In the next slide the
7 effect of ethanol is shown. Again the significant shift from
8 297 to 330 millimicrons. And in the next slide the effect
9 of trichloroacetic acid and benzene is shown. For comparison
10 we give in each case the emission spectrum in cyclohexane,
11 maximum 297. Here there is no appreciable shift but there
12 is the remarkable quenching effect brought about the solvent,
13 in one case benzene and in the other case the presence of
14 one percent trichloroacetic acid.

15 We have examined the
16 theoretical implications of these results as applied to
17 indole and its derivatives and this work now appears in the
18 August issue of the Journal of Organic Chemistry to those of
19 you who are interested. However, I do not believe it
20 pertinent in this discussion except to say that these solvent
21 effects can be discussed in terms of solvent polarity and
22 hydrogen bonding.

23 Now, the fluorescence
24 spectra of alkyl derivatives of aromatic compounds are
25 usually very similar to that of the parent compounds. In

1 the methylindoles shown in the next slide that is not the
2 case. We find there are shifts from 5 to 25 millimicrons
3 from one compound to the next in the same solvent. For
4 example, indole in water shows a maximum at 350 millimicrons,
5 2,3-dimethylindole 376. In dioxane solution indole can be
6 very readily distinguished from the various methylindoles
7 and in cyclohexane 2 and 3 methylindole are readily
8 distinguished.

9 It should be noted that
10 the UV's of all these methylindoles are very similar so
11 that we are provided here with a method by which we can
12 distinguish between these various methylindoles without
13 having to take resort to UV adsorption spectroscopy.

14 The relative fluorescence
15 intensities of some of the methylindoles are compared on
16 the next slide. As you see there is a marked decrease in
17 intensity in benzene and a less marked decrease in intensity
18 in water. In fact, in the 2 and 3 methylindoles the
19 fluorescence in benzene was so weak that the 5 millimicron
20 slits ordinarily used in this work had to be replaced by 10
21 millimicron slits in order to provide more energy, of course.

22 The fluorescence data of
23 1,2-dimethylindole and 1-methyl-2-phenylindole are given
24 in the next slide. Both of these compounds show shift to
25 longer wave length in going from cyclohexane to water while the

1 UV adsorption spectra remain unchanged. 1-methyl-2-
2 phenolindole is remarkable for the much higher fluorescence
3 intensity it exhibits in the various solvents compared to
4 the other compounds in the series.

5 3-Acetylindole, another
6 of the compounds which we have looked at, unlike the other
7 indole derivatives does not show fluorescence in any of the
8 solvents. Its UV adsorption data which we have in the
9 next slide shows a shift to a longer wave length going from
10 solvents of low to solvents of high dielectric constant.
11 This kind of solvent effect of carbonyl compounds has been
12 observed and studied for other ketones.

13 Indole-3-acetic acid
14 shows no measurable fluorescence in cyclohexane and in
15 benzene but it does fluoresce in dioxane, ethanol and water,
16 as we have in the next slide, with shifts to a longer wave
17 length in that order. The values in parentheses, by the way,
18 are literature values. The fluorescence intensities in
19 these solvents are comparable to that of the other indole
20 derivatives and the UV adsorption spectra again, as with the
21 others, remain unchanged.

22 Carbazole does exhibit
23 shifts in fluorescence emission maxima to longer wave lengths
24 with increase in dielectric constant to the solvent. However,
25 these shifts are smaller than in the indoles and in addition

1 comparable shifts are observed in the UV adsorption spectra
2 with change of solvent.

3 The pyridine series was
4 next examined. Pyridine itself, as is well known, does not
5 fluoresce and quinoline when pure, which is quite difficult
6 by the way to purify, fluoresces only weakly.

7 The next higher analog,
8 acridine, does show fluorescence. We have examined the
9 fluorescence of acridine and its angular isomers of
10 3,4,5,6, and 7,8-benzoquinoline. The fluorescence of
11 acridine in various solvents is shown in the next slide.
12 The fluorescence intensity of acridine is remarkably weak
13 in cyclohexane, an emission maxima of 400. Again with
14 change of solvent there is a shift to longer wave length,
15 rather new bands appeared with longer wave length, and the
16 fluorescence intensity increases vastly.

17 In remarkable contrast
18 the angular isomers of acridine, 3,4, 5,6, 7,8-benzoquinoline,
19 did not show any of these solvent effects. Acridine,
20 therefore, occupies an unusual position in this series and
21 this procedure may be used to distinguish acridine from its
22 angular isomers.

23 It is to be expected that
24 the extension of studies of this kind to other aromatics,
25 particularly in the heterocyclic series, will yield information

which can be used for qualitative and quantitative analysis of aromatic air pollutants. We have examined the analysis of mixtures of hydrocarbons by fluorescence which is a subject which I have not yet heard mentioned at this talk. Since this is an area which may be of direct concern I might say a few words as far as the rather exploratory experiments which we carried out and by no means exhaustive.

The fluorescence exhibited by some compounds is so intense as to be distinguishable even in very complex mixtures. This fact has been known for many years and was in fact used by Cook and co-workers in identification and isolation of benzo(a)pyrene from coal tar.

However, the detection of compounds with a lower fluorescence efficiency than benzo(a)pyrene was examined and found difficult. A mixture containing one microgram of each of anthracene, pyrene, fluoranthene, 3,4, 9,10-dibenzopyrene, benzo(a)pyrene and benzo(g,h,i)fluoranthene were prepared. All six compounds purified by paper chromatography before use.

Emission spectra were obtained on this mixture at excitation wave lengths from 250 to 400 millimicrons at 10 millimicron intervals. All the emission spectra from 250 to 300 and from 320 to 390 millimicrons were identical and agreed with the fluorescence emission spectrum of benzo(a)pyrene. The spectra obtained

1 at excitation 310 and at 400 agreed with the emission spectrum
2 of dibenzo(c,d,j,k)pyrene, that is 3,4, 9,10-dibenzopyrene.

3 The other four compounds
4 could not be detected when under conditions used at least
5 in this experiment in the presence of the strongly
6 fluorescence benzo(a)pyrene and 3,4, 9,10-dibenzopyrene.

7 Since several useful
8 methods are available for the paper chromatography of
9 aromatic compounds we did not go further into the examination
10 of this aspect.

11 Now, the fourth and last
12 phase of our work which may be of interest to this seminar
13 concerns an examination of carcinogenic oxy compounds with
14 which I quite unintentionally created so much confusion
15 yesterday. These include the carcinogenic hydroperoxides,
16 peroxides, epoxides and lactones on which only sporadic
17 carcinogenicity tests have been carried out.

18 Kotin has postulated that
19 epoxides and peroxy compounds formed by the oxidation of
20 olefins are at least in part responsible for the
21 carcinogenicity of polluted air and was able to induce
22 pulmonary tumors in mice using ozonised gasoline.

23 The significance of these
24 compounds as possible carcinogenic air pollutants has been
25 stressed by Kotin and by Haagen - Smit on several

occasions. Epoxides, hydroperoxides and peroxides are, of course, particularly suspect since it is conceivable that they will be formed by autoxidation or photochemical oxidation of olefins in the air.

These compounds are of interest to us primarily because the study of their carcinogenicity may yield some information about the early stages in the metabolism of aromatic carcinogens. Thus it has been postulated by Boyland in England that epoxides are intermediates in the formation of the dihydrodiols, the type of structure that I had shown you earlier. Such epoxide intermediates would be of interest if they are indeed formed since carcinogenicity has been reported for diepoxybutane and for vinylcyclohexene dioxide.

Fieser has reported carcinogenicity for sterol hydroperoxide. Next slide, please. This is the partial structure for Fieser's hydroperoxide, it's simply a sterol. In other words, one more ring cycle of pentane in the alkyl side chain. You will notice the characteristic structure feature of this hydroperoxide is that it has the hydroperoxy group, alpha to the carbon-carbon double bond. Fieser had induced carcinomas in rats by subcutaneous injection of this material.

In trying to do this kind
of experiment with simpler compounds we prepared the

1 hydroperoxide of butadiene dimer otherwise called
2 vinylcyclohexene, this compound, destroyed by the dimerization
3 of butadiene. This very simple oxygenation at 70° C.
4 gives in about one percent yield vinylcyclohexene
5 hydroperoxide. This compound we have now tested by skin
6 painting on mice and we were able to induce cancers in 15
7 out of the 30 animals used in the experiment.

8 Not all epoxides or all
9 hydroperoxides are, of course, carcinogenic. 1,2, 4,5-diepoxy
10 pentane in which the two epoxide functions are separated by
11 one methylene group is not carcinogenic according to the
12 work of Walpole. And various epoxides which we have tested
13 are not carcinogenic. These include such compounds as the
14 epoxides of oleic and linoleic acids and squalene-
15 hexaepoxide.

16 Currently we are preparing
17 and testing a series of these compounds for carcinogenic
18 activity. Once structural features which are required to
19 confer carcinogenicity in these compounds are identified the
20 air pollution researchers would be in a better position to
21 examine their products for these materials.

22 The question of detection
23 and assay of such compounds is another matter and since this
24 was brought up yesterday I would like to show you one last
25 slide in which we had examined the epoxidation of

1 1,2-epoxy butene 3 and this is the gas chromatogram of this
2 material. That happens to be the styrene material and this
3 is solvent and these are the two products, the racemic
4 and meso diepox butane. We see that in spite of the
5 fact that these compounds differ only in their stereochemistry
6 they can be separated by gas chromatography. Using
7 preparative gas chromatography we were able to separate these
8 otherwise very difficult purifiable compounds and determine
9 for once and all their characteristic infrared absorption
10 spectra.

11 To those of you who are
12 interested in this area there is also in the literature
13 paper chromatographic methods for the separation of
14 hydroperoxides and peroxides

15 In summary then I have
16 attempted to describe briefly some areas of our research in
17 chemical carcinogenesis which may be of interest to air
18 pollution specialists. These included the identification of
19 aromatic hydrocarbons and heterocyclics from cigarette smoke;
20 the identification and structural determination of oxidation
21 products of dibenzanthracene; some aspects of fluorescence
22 spectroscopy; and finally, the significance of non-aromatic
23 oxy compounds as possible air polluting carcinogens.

24 Thank you.

25 CHAIRMAN SAWICKI: Are there any questions

1 or comments? Dr. Hoffmann.

2 DR. HOFFMANN: Dietrich Hoffmann, Sloan-
3 Kettering Institute, New York City. Dr. Van Duuren, which
4 concentration did you use for your vinylcyclohexene peroxide
5 when you applied it to mouse skin?

6 DR. VAN DUUREN: It was one-half of one
7 percent in benzene.

8 DR. HOFFMANN: Thank you, very much.
9 Dr. Van Duuren, these fluorescence studies of indole derivatives,
10 are they used in the study of tryptophane metabolism? This
11 would be an enormous tool.

12 DR. VAN DUUREN: Yes, it's nice here to
13 bring that up. I would hope that this kind of work would
14 be of value to people who are in that kind of research, and
15 I know that very much of it is going on at the NIH.

16 DR. HOFFMANN: I have one question more
17 and this is, Dr. Van Duuren, you isolated the dihydroxy
18 dibenz(a,h)anthracene and you identified it by reaction
19 and finally a methoxy derivative. Do you think this methoxy
20 derivative might be present as one metabolism product of
21 dibenz(a,h)anthracene since Schoental and Berenblum proved
22 a high carcinogenic activity of 8-methoxy benzo(a)pyrene?
23 I heard recently a paper given by Dr. Berenblum and he thinks
24 that this might be as metabolism products strong carcinogens.

25 DR. VAN DUUREN: From the point of view

1 of mechanism of formation I can't see any reason why it
2 should be formed metabolically. I don't believe that any
3 of these ethers have been found there. Of course, these
4 compounds do occur as conjugates, glucuronides, sulfates,
5 phosphates, what have you. Dr. Falk may have a comment on
6 that.

7 DR. FALK: Falk, UC. I read that
8 paper and obviously looked for the three hydroxy compound,
9 and first of all, in the bile of a rat there is not a trace
10 of 3-hydroxy benzopyrene found. This compound is later on
11 created by hydrolysis of the glucuronide conjugate and there
12 was not a trace of the methoxy present even though I looked
13 for it high and low.

14 CHAIRMAN SAWICKI: Dr. Badger?

15 DR. BADGER: Badger, Australia. I
16 wonder if I may ask whether the configuration of the diol of
17 dibenzanthracene was determined. The cis-diol is, of course,
18 obtainable synthetically from the hydrocarbons plus osmium
19 tetroxide.

20 DR. VAN DUUREN: Yes, there is maybe one
21 point that I did not stress enough and that is that we have
22 been working here with microgram and sub-microgram levels of
23 material. It would be very interesting to know whether it
24 is a cis or the trans compound and as soon as we can get
25 enough material we will put it in the infrared machine and
that certainly would tell us.

1 CHAIRMAN SAWICKI: Dr. Falk?

2 DR. FALK: I wanted to ask you. I
3 somehow didn't quite realize, did you use mono epoxides for
4 your carcinogenic testing or only diepoxides?

5 DR. VAN DUUREN: The epoxides which we
6 have tested were linoleic and stearic which are mono epoxides
7 and squalene hexaepoxide in which the epoxides are one, four
8 positions from each other.

9 DR. FALK: And did both groups
10 produce tumors?

11 DR. VAN DUUREN: None of them did. But
12 there is reported carcinogenicity for the diepoxy butanes
13 and for vinylcyclohexene dioxide.

14 DR. FALK: Yes, we produced tumors
15 with all of them, I mean with the diepoxides or with the
16 mono epoxides that we tested by injection in mice.

17 DR. VAN DUUREN: I see.

18 CHAIRMAN SAWICKI: Any further questions?
19 Thank you, Dr. Van Duuren.

20
21 I see we are ahead of
22 time so. You can relax and talk amongst yourselves and
23 we'll try to contact the photographers. They were supposed
24 to show about 12:15 to 12:30 so we'll try to contact them.

25 (Whereupon, at 12:20 o'clock p. m., the
meeting was adjourned.)

AFTERNOON SESSION

1:45 p. m.

CHAIRMAN SAWICKI: The first speaker for this session has had a Bachelor's Degree from McGill University and a Masters from the same university of Montreal and a Ph.D. in 1929 from McGill University.

He has been a consultant in air pollution. He started publishing right after he got his Bachelor's Degree. He has worked on determination of carbon monoxide, sulfur dioxide, nitron oxides and he has had many articles on air pollution in literature.

He has worked on identification of benzopyrene, pyrene and other polycyclic hydrocarbons in the air of railway tunnels and the atmosphere itself. He has done pollution studies in our Detroit and Windsor area. More recently he has undertaken the direction of the environmental assessment unit of the Occupational Health Division, Department of National Health and Welfare in Canada. Dr. Katz.

DR. KATZ: My paper deals with some problems concerning the analysis of polycyclics and this is based on the work done with my colleagues, Mr. Monkman and Mr. Moore and Mr. Dubois. Since the earlier British work of Wedgwood and Cooper and others the instrumentation available for the identification of potential carcinogens in the atmosphere has improved greatly.

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analytical manipulation, the practical requirements of those responsible for the assessment of biological activity of airborne hydrocarbons or groups of hydrocarbons and questions regarding the atmospheric reactions which modify or eliminate the airborne polynuclears, all require investigation.

There are essential differences between the particulate loading of the atmosphere in North America and in the United Kingdom or other parts of Europe. For example, Commins has reported an average dust loading over a six months' interval of more than 900 micrograms per cubic meter of air in Liverpool, certain station in Liverpool. In the United States and Canada an average particulate load of about 200 micrograms per cubic meter would be considered high.

To collect comparable weights of solids, greater volumes of air must, therefore, be sampled here than in the United Kingdom. Also, the combustion of coal for domestic heating is a relatively greater source of pollution in the United Kingdom and Europe than in North America. These two factors can be assumed to change the basic patterns of the problem in both North America, and in Europe.

For example, common experience shows that the volatility of naphthalene, anthracene and other members of the polycyclic group is

1 substantial at temperatures far below their melting points.
2 It is likely, therefore, that the aspiration of large volumes
3 of air through fibreglass sheets results in low and
4 indeterminate collection efficiencies for the various
5 hydrocarbons, and that comparisons of concentration data are
6 subject to a high degree of qualification.

7 In Canada air pollution
8 problems are in general less acute than in the United
9 Kingdom and the United States. The very large concentrations
10 of population such as London, New York and Los Angeles are
11 not a feature of the country. However, steam locomotives
12 have been entirely eliminated from the railways of Canada
13 and coal as a domestic fuel has been to a large extent
14 replaced by fuel oil and gas.

15 Also, heavy concentrations
16 of industry, increasing traffic density in moderate sized
17 cities, local topographical and climatological features,
18 and other variables result in problems which are comparable
19 with those in the United States and to a certain extent in
20 the United Kingdom.

21 With regard specifically
22 to atmospheric carcinogens, staff limitations have permitted
23 only a relatively modest effort in Canada. However, Shore
24 and Katz, Moore and Katz and Dubois-Monkman have reported on
25 some eight or more polycyclics found in urban atmospheres,

1 as well as the concentrations in some of these.

2 A number of larger Canadian
3 cities are cooperating in the collection of particulate
4 samples in a manner suited to the determination of polynuclears,
5 that is, without heating the samples in any way and doing
6 other things which will insure that the samples have not
7 lost a great deal of their polycyclics in collection
8 handling. Advanced instrumentation will also permit the
9 investigation of recently formulated ideas on the subject.

10 It would seem that the
11 time is opportune for an objective analysis of previous work
12 in the field and for discussion of objectives, methods and
13 programs for future work. Before proceeding, therefore, to
14 a detailed description of some of the problems encountered
15 in our work, it is worthwhile to mention our recent experience
16 with some of the newer techniques.

17 Thin layer chromatography
18 has been investigated to a point where it appears that it
19 might be applicable to the analysis of the neutral aromatics
20 and might be useful for preparative purposes. Vapor phase
21 chromatography has been investigated somewhat more extensively.

22 However, problems with
23 regard to preparation and injection of the sample, choice of
24 solid support and stationary phase in the column and choice
25 of detector have not been resolved. Phosphorimetry has not

1 yet been investigated experimentally in this laboratory. We
2 think that these, and other approaches, merit an exchange of
3 ideas and the expression of speculative opinion.

4 Some of the problems to be
5 discussed in this paper will deal with the volatility of
6 polynuclears; incomplete resolution on columns; degradation
7 by exposure to ultraviolet radiation; difficulties in
8 interpretation; shortcomings of sampling techniques; solvent
9 effects on fluorescence; the ambiguity of fluorescent
10 intensity measurements; non-reproducibility of R_f values in
11 paper chromatography; contamination and irregularity of
12 acetylated paper; and unsatisfactory purity of reference
13 standards.

14 I don't mean to say I am
15 going to deal exhaustively with each one of these but to
16 indicate in a general way that these factors must be taken
17 into account when you are dealing with actual air pollution
18 samples.

19 Now, with regard to the
20 volatility of polynuclears. This, of course, is fairly well
21 known but I would like to have the first slide please.
22 This shows the ultraviolet absorption spectrum of a three
23 millimeter iso-octane extract from a diesel exhaust sample.
24 This was prepared from the collection of exhaust particulates
25 during the idling phase of a 1750 horsepower diesel and the

material was brownish and greasy, and benzene extraction showed a low insoluble content. Most of the material was soluble in benzene, in other words.

The fiberglass sheets used for collection were extracted with benzene within two weeks of sampling and were not heated in any way to dry the samples. A conventional fractionation was carried out on a silica gel column into aliphatic, aromatic and oxygenated fractions. Qualitatively, the absorption peaks illustrated seem to indicate naphthalene or alkyl naphthalene. So that is indicative of naphthalene and alkyl naphthalenes.

Three points with regard to this curve are worth noting. Firstly, the presence of naphthalene in an undried diesel exhaust sample leads to the surmise that even brief oven drying of air particulate samples changes the quality of the extractables significantly. This is, of course, if diesel exhausts are prevalent in the atmosphere along with automobile exhausts and other products.

Secondly, the finding of naphthalene in the supposedly aliphatic fraction indicates that fractionation on a silica gel column is not exact with respect to the resolution of aliphatic, aromatic and oxygenated groups. We had previously come to this conclusion when with a sufficient volume of iso-octane it was found possible to elute pyrene from air samples. The indicated course of

1 future work is either to refine the method of group
2 separation or to replace it with something else.

3 Thirdly, the identification
4 as naphthalene, of the material whose spectrum is shown, is
5 obviously open to question. Supplementary fluorimetric curves
6 helped to identify the material as probably naphthalene or a
7 naphthalene derivative, but neither ultraviolet absorption,
8 nor fluorescence, nor both together could exactly identify the
9 substance.

10 The importance of naphthalene
11 as a constituent of the polynuclears is perhaps trivial. It
12 does serve to point out that losses of the more volatile
13 polycyclics can be lessened during sampling and subsequent
14 treatment.

15 In this connection, Commins
16 and Lawther discuss 3,4-benzopyrene losses due to volatility
17 during sampling, and Monkman and Dubois indicate the
18 possibility of anthracene losses during paper chromatography.
19 More generally, consideration of the analytical difficulties
20 met with in such a relatively simple case emphasizes the
21 troubles to be expected with the more complex polynuclear
22 hydrocarbons. Combustion processes are evidently not well
23 adapted to the production of pure hydrocarbons for analytical
24 purposes.

25 In the case of naphthalene,

1 it seems reasonable to expect that the parent substance might
2 be associated with varying quantities of alkyl derivatives,
3 hydro derivatives, and incipiently oxygenated products.
4 Some, or all of these constituents are sufficient to shift
5 the diagnostic ultraviolet absorption peaks a few
6 millimicrons, or enough to make positive identification
7 doubtful. In our limited experience with the fluorimetry
8 of air samples, similar remarks would seem to apply to
9 fluorescence spectra.

10 We now come to an example
11 of incomplete resolution which is shown in the ultraviolet
12 absorption spectrum of a composite of cyclohexane fractions
13 from a column in Figure 2. The pyrene structure predominates.
14 Measurement of the 334 millimicron peak indicated 29
15 micrograms of pyrene in the sample. The peak at 286
16 millimicrons represents about 17 micrograms of fluoranthene.

17 Then we'll show you Figure
18 3 which is the spectrum of a composite of elution fractions
19 showing decreasing amounts of fluoranthene. Here there is
20 still pyrene present and the concentrations there were 4.5
21 and 13 micrograms.

22 These two curves
23 illustrate the difficulty of resolving pyrene and fluoranthene
24 on a column. These materials were derived from about 9000
25 cubic meters of Ottawa air sampled in the middle of March,

1961. Ottawa has a population of about 260,000 and it isn't what you would call a heavily industrialized city. There is only light industry present.

The estimated dust loading of particulates during this sampling was about 68 micrograms per cubic meter of air. On this basis, adding up the total pyrene and fluoranthene, the pyrene concentration was 0.37 micrograms per 100 cubic meters of air and that of fluoranthene was 0.33 micrograms per 100 cubic meter of air. Now, remember that we had to sample about 300,000 cubic feet of air to get these samples.

These two curves illustrate the difficulty of resolving pyrene and fluoranthene on a column. It is relatively easy to select small elution fractions, as column chromatography progresses, which show predominant structure for either pyrene or fluoranthene with only trace indications of one or the other. When quantitative estimation is required, however, the limit of practicability, we believe, is about as shown.

Now, a not inconsiderable difficulty has been indicated in the liquid chromatographic analysis of pyrene and fluoranthene. It might also be mentioned that these isomers do not separate any better by paper chromatography. The best method of distinguishing or determining pyrene and fluoranthene in liquid or paper

1 chromatograms is probably fluorescence spectroscopy of the
2 two-component mixture.

3 At suitable actuation wave
4 lengths the individual fluorescence spectra can be obtained.
5 This is illustrated in Figure 4. This shows the separation
6 of the two components, pyrene and fluoranthene, and this is
7 the mixture here.

8 With regard to degradation
9 by ultraviolet light, a composite sample representing
10 predominantly chrysene was taken up on alumina and added to
11 a silica gel/cyclohexane column. Elution was carried out
12 under continuous exposure to ultraviolet irradiation. The
13 ultraviolet spectrum of the eluate removable from the column
14 with cyclohexane plus benzene is shown in the next slide.
15 The solid line represents the mixture which contained
16 predominately chrysene, and the dotted curve, this one here,
17 indicates the ultraviolet absorption after irradiation under
18 ultraviolet light.

19 The spectrum was determined
20 in iso-octane and shows a slight shifting of peaks but
21 essentially the same structure as Curve I. That is Curve II
22 shows essentially the same structure. Curve III is the
23 spectrum in iso-octane of a fraction removable from the
24 column with alcohol. This was a sharply defined brown yellow
25 zone only partially soluble in iso-octane.

1 The results suggest the
2 usefulness of a quantitative repetition of the work with a
3 carefully purified sample of chrysene in quantity sufficient
4 to permit investigation of this brown-yellow background
5 material, which is illustrated by Curve III.

6 The preceding results are
7 in agreement with Miller and Baumann's work who found that a
8 group of six polynuclears, including 3,4-benzopyrene, were
9 destroyed by ultraviolet radiation. They did not carry out
10 any work with chrysene. On the other handk they found that
11 dibenzanthracene was not destroyed by ultraviolet light.
12 Obviously, care must be taken to avoid undue exposure to
13 ultraviolet light during sampling, extraction and measurement.

14 In the next slide we have
15 a spectrum in iso-octane of a fraction which might best be
16 described as probably containing 3,4-benzopyrene structure,
17 and then by re-chromatographing on somewhat less active
18 alumina and following that with another successive
19 re-chromatographing the curve shown on the solid line was
20 obtained on ultraviolet absorption.

21 The sequence of
22 manipulations necessary to prepare a spectrum which is still
23 less than definitive is, in our opinion, too involved to be
24 practical. Our main purpose in showing the curve is to
25 demonstrate the difficulty of getting even ambiguous results

1 with small concentrations of 3,4-benzopyrene. Now, I don't
2 mean this applies to samples where 3,4-benzopyrene might be
3 plentiful and difficulties in interpretation would be
4 correspondingly less.

5 One might remark with some
6 justification that many papers on the subject are notable for
7 their apparent reluctance to reproduce the curves from which
8 the reported 3,4-benzopyrene concentrations are derived. The
9 foregoing is an example of the troubles encountered in the
10 analysis of air samples, by methods which are described as
11 simple, rapid and accurate.

12 We believe that such
13 methods, based on the analytical isolation of the components
14 of synthetic mixtures of pure constituents or tests assumed
15 to be specific in the presence of interferences leave much
16 to be desired.

17 The question of the so-
18 called background material is in some cases largely ignored.
19 This material appears, from ultraviolet absorption curves,
20 to be present in greater quantity than the materials looked
21 for. Until the nature of the background material is known
22 with more certainty, the exact analysis of air samples by
23 ultraviolet absorption is seriously handicapped.

24 Our impression from
25 preliminary experience with fluorescence is that fluorometric

1 methods may add a degree of certainty to the interpretation
2 of ultraviolet absorption data, but that either or both are
3 not completely definitive.

4 With regard to sampling
5 and analysis, we have indicated that some of the current
6 sampling and testing methods may not be satisfactory. We
7 believe that too much emphasis is placed on samples taken on
8 glass fiber sheets. This is convenient but it is probable
9 that sampling efficiency is not the same for all polynuclears.
10 The loading effect, and the unevenness of sample deposition
11 are well known.

12 The adverse effects of
13 heat in the drying of glass fiber samples to constant weight
14 has been mentioned. In the past, conventional treatment of
15 the sample has involved exposure to heat, air, and
16 ultraviolet light. This has resulted in numerous air
17 sample analyses, in which the predominant polynuclear was
18 coronene, with little evidence of anything else. This is
19 probably due to the lower volatility and the greater stability
20 of coronene as compared with others of the group.

21 Sampling in a liquid
22 absorbent possesses the advantage of eliminating subsequent
23 extraction and speeding up the overall process. It is
24 simpler and more direct and should be free from the adsorption
25 effects associated with carbonaceous material caught on the

1 glass fiber at the same time.

2 In this connection,
3 Balgairies and Claeys suggest sampling by aspirating air
4 through purified spectrograde cyclohexane and measuring the
5 fluorescence at a selected wave length. The value obtained
6 is called the anthracene index and is a representation of the
7 fluorescent intensity of 100 ml cyclohexane through which has
8 been aspirated 100 litres of air. It is reported that 0.0001
9 micrograms of anthracene per ml gives a measurable
10 fluorescence. Sawicki et al report a K_Q value of 23.1 at
11 380 millimicrons for 3,4-benzopyrene.

12 Balgairies and Claeys use
13 the 3865 angstrom mercury line for excitation. With the
14 stronger excitation and continuous energy now available in
15 the new Xenon arc lamps and with the flexibility of some of
16 the new instruments it is apparent that much improvement may
17 be made in sensitivity and specificity.

18 Preliminary experiments with
19 the above sampling technique were made on consecutive air
20 samples aspirated in cyclohexane at the same source.
21 Fluorescence spectra were determined directly as the sample
22 was removed from the bubbler. It was evident from the spectra
23 obtained that the air sample had a distinctive pattern which
24 was constant for the duration of sampling.

25 Column chromatography of

1 the cyclohexane samples resulted in the fluorescence curves
2 shown in Figure 7. This is a composite of four samples,
3 eluates, and this gives the fluorescence spectra at various
4 wave lengths.

5
6 In the next slide, these
7 are the various zones, 1 and 2, and then an authentic sample
8 of anthracene is shown 3 here and you have the excitation
9 wave lengths indicated. The preliminary spectra were obtained
10 minutes after receipt of the sample. The separation necessary
11 to produce the other curves required only two hours. One
12 of the predominant hydrocarbons would seem to be anthracene.

13
14 Now, in the next slide we
15 will show you an emission spectra of a composite column
16 eluate of the air sample in which the anthracene structure
17 is definitely indicated in the emission spectrum.

18
19 With regard to some
20 solvent effects on fluorescence it is found in quantitative
21 work there are intensity losses due to concentration quenching,
22 localisation of fluorescence and self absorption. These
23 effects may be decreased by making the measurements on
24 sufficiently dilute solutions. As a practical criterion for
25 this purpose Chaudet and Kaye adjust the ultraviolet
absorbance of the solution to 0.015 per centimeters, measured
at 365 millimicrons, which results in concentrations about
 1×10^{-6} M. The concentrations suggested by Sawicki are of the

order of 1×10^{-5} M.

According to Miller and Baumann fluorescence is inhibited by dissolved oxygen. They found that the fluorescence intensities of hydrocarbons such as 3,4-benzpyrene varied hyperbolically with the partial pressure of oxygen. It is customary to remove oxygen from the solution with a stream of nitrogen before measurement. Likewise, the sample may be evacuated to remove dissolved oxygen.

The solvent used has its effect, the fluorescence of 3,4-benzpyrene in ethyl ether, for example, being double the fluorescence in petroleum ether, for the same concentration of 3,4-benzpyrene and oxygen.

Tetrahydrofurfuryl alcohol, pyridine, and dioxane dissolve comparatively small amounts of oxygen and show large fluorescence intensities in the presence of air. These three solvents, incidentally, contain ether-type linkages. Nitrobenzene dissolves little oxygen but it completely inhibits the fluorescence of the hydrocarbons.

nitration of 12,2-benzanthracene to the mono-nitro derivative results in the complete loss of fluorescence. Sulphur dioxide is a much more potent inhibitor of fluorescence than oxygen and the fluorescence of 3,4-benzpyrene is completely and

reversibly inhibited in concentrations of 10 micrograms per ml when the solvent is saturated with sulphur dioxide at 740 millimeters pressure.

The choice of the best solvent for fluorescence work may have to be a compromise. It should be free from fluorescence or easily made so. Preferably also it should be transparent in the ultraviolet to allow application of both ultraviolet absorption and fluorescence measurements. If possible, it should have a low affinity for oxygen but a high solubility for polycyclic hydrocarbons. It should be stable and neither react with nor inhibit its fluorescence.

Pyridine, though a desirable solvent, cannot be produced without fluorescence. Cyclohexane is not a particularly good solvent for polynuclear hydrocarbons. Chlorinated and nitro compounds are unsuitable. The former in the presence of ultraviolet radiation rapidly destroy the fluorescence of the hydrocarbon. This suggests that the use of methylene chloride as a preliminary extractant in the processing of glass fiber samples may be desirable.

With regard to the relative intensity of fluorescence, it is well known the intensity of fluorescence varies with the particular hydrocarbon. 0.001 microgram of 3,4-benzpyrene in the form of a chromatogram spot is easily visible to the eye under ultraviolet

1 in the solid and liquid states.

2 With regard to paper
3 chromatography, up to the present time we have had difficulty
4 in obtaining reproducible R_f values. Using acetylated paper,
5 reproducibility within the sheet, from sheet to sheet, and
6 from batch to batch, leaves much to be desired.

7 It is suggested that the
8 paper should be acetylated and washed in the fibre stage.
9 This should permit the preparation of a uniformly acetylated
10 fibre. The paper sheets could then be prepared from the raw
11 material. Improvement should result as regards control of
12 degree of acetylation, and uniformity and cleanliness of the
13 finished sheets.

14 In the preceding we
15 mentioned our hope of achieving some consistency in R_f
16 measurement. Separations, though not perfect, are in a
17 better position. In the case of air samples the multitude
18 of zones present and the tendency toward a streaking
19 fluorescent background result in a very complex and crowded
20 chromatograms. With the more instruments now available for
21 identification it is hoped to be able to lessen the crowding
22 by using smaller sample aliquots.

23 Paper chromatography
24 remains, at present, a valuable separation technique. For
25 example, 3,4-benzopyrene and 1,12-benzoperylene, considered

difficult to separate are easily separated on paper.

May I have the next slide, please. Now, you will notice that in cyclohexane solutions the ultraviolet absorption of these two substances is very similar except for this peak for the 3,4-benzopyrene which is missing in the 1,12-benzperylene. Of course, if the concentrations are such that this peak is virtually absent then there is no way with ultraviolet absorption of determining whether you have 3,4-benzopyrene or 12,12-benzperylene.

Ultraviolet absorption or fluorescence measurements applied to chromatogram spots suffer from high background and scatter. It is hoped to be able to lessen the interference by the use of new media with lower backgrounds, and by appropriate treatment of the paper.

A new instrumental approach is suggested such as X-ray diffraction analysis of a paper chromatogram spot. We have under construction a photomultiplier photometer, which we hope will permit total relative fluorescence intensity measurements to be made on paper spots.

I have here a section on our experience with gas chromatography but I will skip most of it except to indicate that one of the problems of gas chromatography is the method of properly introducing samples represented either by particulate samples or other samples

that represent these higher hydrocarbons.

It must be remembered that if the air sample is passed through material such as silica gel "swamping" of the peaks of interest may occur due to the large quantity of water which is likely to be present. And this, of course, suggests various expedients such as a predryer in the sampling train, and a dual function column with the first section consisting of a water vapor absorbent.

Now, the thermal conductivity detectors are being replaced largely by the ionisation detectors which have become very popular, although it is pointed out that the flame ionisation detector does not respond to water vapour and, therefore, possesses a distinct advantage with certain air samples.

The detector cells are likely to be hard to clean and the connections usually have too many bends, and in one case we had to improve the exit tubing from the detector of a commercially available instrument by eliminating 10 right angle bends in a space of perhaps 7 horizontal inches.

We anticipate that gas chromatography will become, eventually, a useful method for polycyclics but there are a number of disadvantages at present. One of these is the method with any detector is essentially non-specific, and one would have to have some means of

1 downstream identification of fractions at the main detector
2 exit using appropriate chemical or physical means to obtain
3 some specificity of the response.

4 And, finally, a plea is
5 addressed to instrument makers in general in that they do not
6 seem to have the special requirements of air pollution
7 analysis in mind. It is hoped that this may be changed to
8 permit the full application of both gas chromatography and
9 other techniques.

10 In summarizing all this,
11 we may say that with regard to available polycyclic hydrocarbons,
12 many of those that can be purchased have been shown to be
13 impure and it's important that this situation be improved.
14 We have found it necessary to prepare some of our standards.
15 This procedure, at present, cannot easily be avoided, but only
16 microgram amounts of a pure standard can be prepared in this
17 way. More work is required to raise the purity of the
18 standards, since all the analytical techniques are based
19 generally on a pure reference standard. In particular, it is
20 desirable to interest the chemical supply houses in preparing
21 such polycyclics to the higher standards of purity required.

22 Thank you.

23 CHAIRMAN SAWICKI: Any questions or comments?

24 DR. VAN DUUREN: Van Duuren, New York
25 University. On behalf of those of us who have been very

1 laboriously separating aromatic hydrocarbons from environmental
2 sources, I would like to take strong exception to Dr. Katz's
3 statement concerning the publication of spectra. There are
4 in the literature and there have been for some years some
5 very excellent spectra from various laboratories.

6 The second point which I
7 would like to comment on is on the separability of pyrene
8 and fluoranthene. These two compounds can, indeed, be
9 separated very nicely by paper chromatography. The impurity
10 in chrysene which has been talked about has been identified
11 by Buhoy (phonetic) and published last year, it turns out
12 to be a linear benzcarbazole. Thank you.

13 CHAIRMAN SAWICKI: You can easily see that
14 impurity if you dissolve that material in dimethylformamide
15 and add alkali, form an alkaline solution, and you get a
16 brilliant yellow fluorescence color and that's due to
17 benzcarbazole. Do you have any comment?

18 DR. KATZ: No comment.

19 CHAIRMAN SAWICKI: Any other questions or
20 comments? Dr. Hoffman?

21 DR. HOFFMAN: Hoffman, Sloan-Kettering
22 Institute, New York City. I agree fully with Dr. Van Duuren
23 and I think when I see his work done on cigarette smoke
24 condensate and published in two excellent publications this
25 mentioning really takes not place and is not the right

1 expression to this and I think this applies also very much
2 to our comments, to Dr. Lyons and to gentlemen working for
3 years in this field. Now, when we didn't show slides, I
4 have a full box of all components which were mentioned. In
5 the slides we showed the ultraviolet spectra. I am very
6 pleased to show them but I think I would bore a lot of the
7 gentlemen here because I think by the time people know the
8 work of others and you have not to present every ultraviolet
9 absorption spectrum. Now, we can present this so it's not
10 our idea not to present spectra because we don't have them.
11 The reason is this, we think we progressed one step further
12 and discussed things where so well experienced people as they
13 are here we can discuss and can learn more, because we think --
14 or let's see, I shouldn't say we, I think we are over the
15 step to discuss UV, ultraviolet adsorption spectra, I think
16 we are one step further.

17 CHAIRMAN SAWICKI: Any other questions?

18 DR. BADGER: Badger, Australia. I would
19 like to ask one question in connection with the acetylated
20 paper. I wasn't quite sure whether Dr. Katz said that you
21 might get a better paper if you acetylated the pulp and then
22 made the paper or whether in fact you do get a better paper.

23 DR. KATZ: We haven't solved this
24 problem of the acetylated paper to our satisfaction. We've
25 suggested to the manufacturers that they should acetylate

1 the pulp, at least try and get a more uniform acetylated
2 paper than the present samples that are being turned out.

3 DR. BADGER: It would be interesting
4 to see that when it comes out. But I would like to draw
5 attention to the paper published by Beckwith and Thompson
6 last year on the phenylation of phenanthrene where they
7 provided a test to check whether your acetylated cellulose
8 was good or not. The test involves a standard column and
9 the separation is from perylene and benzopyrene and under
10 the conditions that they state you should be able to get
11 a seven inch separation of the bands.

12 CHAIRMAN SAWICKI: Any other questions or
13 comments? Thank you, Dr. Katz.

14 We will have a recess and
15 come back at 2:45, but first I would like to make a few
16 announcements.

17 (Short recess.)

18 CHAIRMAN SAWICKI: Our next speaker for this
19 part of the session is Dr. Carugno from Italy. He obtained
20 his Doctorate in 1949 from the University of Rome and he has
21 been in the field of high-temperature gas chromatography ,
22 especially of polynuclear aromatic hydrocarbons. Professor
23 Marni of the University of Cincinnati will give the talk for
24 Dr. Carugno who will be standing by or sitting over there.
25 Professor Marni.

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In order to extend the

research in this field, the high temperature gas-liquid chromatography was deemed to be a suitable means of analysis for the paraffins of tobacco and tobacco smoke. This method was already used with other types of paraffins.

The apparatus "Fractovap"

Model B- Carlo Erba - has been used with a thermal conductivity cell and the apparatus "Fractovap" model C with hydrogen flame ionization detector. The model B is provided with an attachment for high temperatures which includes a device with --

Now, in this case sometimes I will give you the name of the chemical, sometimes I will give you the abbreviated form. This would be carbon dioxide, CO₂, right? Good. So then model B is provided with an attachment for high temperatures which includes a device for the carbon dioxide oxidation of the substances previously chromatographed.

The columns used in the device for high temperatures are made of copper pipe with a millimeter inside diameter, filled with Celite B.D.H. 60-80 mesh as the solid support and Apieson L 10% and 15% as the stationary phase; the columns for the apparatus Model C are of stainless steel filled with Chromosorb W.80-100 mesh as the solid support and Apieson L 10% as the stationary phase.

The pure substances used as standard are specified below: Tridecane, Ligh; Octadecane, Fluka; Docosane, Ligh; Octacosane, Eastman Kodak; Dotriacontane, Eastman Kodak.

1 For the preparation of
2 the analysis materials we have followed, with some modifications
3 the methods of Cusin and others and of Stedman and Rusaniwsky.

4 Now, Step A: After 650
5 grams of tobacco similar to a blend normally used for
6 manufacturing a standard brand of Italian cigarettes are
7 extracted in a Soxhlet apparatus by benzene, we performed the
8 following operation: the neutral phase was separated from
9 the alkaline and acid phase; the residue was extracted by
10 means of boiling ethyl alcohol, and the solution was cooled
11 to -50°C ; then two recrystallizations were done in ethyl
12 alcohol, and the residue of the second was picked up in a
13 cyclohexane and passed through an alumina column as described
14 by Cusin; but the extracted paraffins were not passed through
15 a charcoal column, instead they were put through two recrystal-
16 lizations in ethyl alcohol. In this way a perfectly white
17 product was obtained.

18 Now, Step B: 370 grams
19 of the same tobacco blend were extracted in a Soxhlet
20 apparatus with 750 milliliters of n-hexane for 25 hours,
21 and this was followed by the process proposed by Stedman
22 except that at the end there were added two recrystallizations
23 in ethyl alcohol at -14°C . With this process also perfectly
24 white paraffins.

25 1500 cigarettes manufactured

1 with a second lot of the tobacco used in the above-mentioned
2 tests, are smoked with an automatic machine under standard
3 conditions. Interval between puffs: 60 seconds; duration
4 of a puff: 2 seconds; volume of a puff: 35 milliliters.

5 The condensate of the smoke
6 is dissolved in 500 milliliters of methanol, the solvent
7 is evaporated and the residue is dissolved in benzol and
8 n-hexane, respectively.

9 The subsequent processes
10 are analogous to those used for the tobacco paraffins
11 except that in the second a further chromatography on an
12 alumina column is carried out.

13 The equation is done with
14 250 milliliters of cyclohexane, the solvent is removed in a
15 steam bath and the residue is reobtained with ethanol. In
16 this case two crystallizations at -14°C are performed.

17 Now, analysis. Now, Slide
18 1, please. Now, speaking about the slides, I am not prepared
19 to discuss the slides. I shall put them on the screen. I
20 shall read what it says and I hope that you will follow by
21 yourselves what they tell.

22 The analyses were carried
23 out under the following conditions: A one meter column of
24 15 percent Apieson L as a stationary phase on a Celite 60-80
25 mesh as solid support. Injection temperature 400°C .

1 Temperature of the column 230°C. Temperature of the combustion
2 furnace 750°C. Temperature of the thermal conductivity cell
3 (thermistors) 5°C. Carrier gas: Helium at 24 milliliters
4 per minute. Inlet pressure 456 millimeters of mercury.
5 Outlet pressure: atmospheric. Analysis time: from 65-70
6 minutes.

7
8 Now, I will give you a
9 moment to look over the slide. Now, then, Slide 2, please.
10 A one meter column of 10 percent Apieson L as stationary
11 phase on Celite 60-80 mesh as solid support. Injection
12 temperature: 400°C. Column temperature: 280°C. Temperature
13 of the combustion furnace: 750°C. Temperature of the
14 thermal conductivity cell: 5°C. Carrier gas: Helium at 31
15 milliliters per minute. Inlet pressure: 532 millimeters
16 of mercury. Outlet pressure: atmospheric. Analysis time:
17 30 minutes. Again I will give you a few moments.

18 Slide 3, please. A one
19 meter column of 10 percent Apieson L as stationary phase on
20 Chromosorb W 80-100 mesh as solid support. Injection temperature:
21 360°C. Column temperature: 230°C. Detector: flame
22 ionisation detector. Carrier gas: nitrogen at 18 milliliters
23 per minute. Inlet pressure: 684 millimeters of mercury.
24 Outlet pressure: atmospheric. Analysis time: 53 minutes.

25 Slide 4. A one meter
column of 10 percent Apieson L as stationary phase on

1 Chromosorb W. 80-100 mesh as solid support. Injection
2 temperature: 390°C. Column temperature: 255°C. Detector:
3 flame ionization detector. Carrier gas: Nitrogen at 25
4 milliliters per minute. Inlet pressure: 852 millimeters of
5 mercury. Outlet pressure: atmospheric. Analysis time:
6 173 minutes.

7
8 Slide 5. A one meter
9 column of 10 percent Apieson L as stationary phase on
10 Chromosorb W. 80-100 mesh as solid support. Injection
11 temperature: 280°C. Column temperature: 253°C. Detector:
12 flame ionization detector. Carrier gas: Nitrogen at 25
13 milliliters per minute. Inlet pressure 852 millimeters of
14 mercury. Outlet pressure: atmospheric. Analysis time:
15 15 minutes.

16 The paraffin samples were
17 brought to melting temperature and then introduced into the
18 apparatus with a Hamilton syringe.

19 The sample size is from
20 0.02 to 0.05 milliliters working with a high temperature
21 apparatus with a thermal conductivity cell, and of about
22 0.05 microliters when a flame ionization detector model is
23 used.

24 Results and discussion.

25 In Slides 1 and 2 has been shown the chromatograms obtained
by means of a high temperature auxiliary while in Slides 4

1 and 5 have been given the chromatograms obtained with an
2 apparatus with a flame ionization detector. The condition
3 of analysis has been described above.

4 Confirming the complexity
5 of the composition of paraffins extracted from tobacco and
6 tobacco smoke, it was possible, by means of gas chromatography
7 to ascertain the presence of paraffins of a very low melting
8 point and of liquid paraffins at ordinary temperatures.
9 They correspond to hydrocarbons having a low number of
10 carbon atoms starting from C₈.

11 Now, slide 3 again, please.

12 In particular, the analysis of the tobacco paraffins was
13 achieved by working at a lower temperature, namely, 230°C
14 and by exploiting the high sensitivity obtained with the
15 flame ionization detector. Furthermore, the presence of
16 considerable quantities of iso-paraffins was useful, and
17 in this connection it must be noted that in all the analyses
18 carried out the peaks corresponding to C₃₀ and to C₃₂ do not
19 consist mainly of n-paraffins. To confirm this, the
20 identification of peaks has been based on the data of retention
21 times of some pure compounds as docosane, octasane and
22 dotriacontane.

23 Slide 6 again, please.

24 The retention time of all n-paraffins has been determined,
25 and the relation between the logarithm of the retention

1 volume and the number of carbon atoms has been verified.
2 A straight line was obtained, as you see there.

3 Slide 7, please. We
4 have also tried to verify if this linearity exists also
5 for the iso-paraffins from C₂₇ to C₃₈. This slide gives this
6 confirmation.

7 Now, slide 5 again. In
8 the chromatogram of the paraffins of tobacco smoke a smeared
9 background may be observed under the peaks; this probably
10 may be due to the presence of foreign substances not fully
11 separated in the crystallization phase. Furthermore, the
12 absence of paraffinic hydrocarbons from C₁₄ to C₈ has been
13 noted. On the other hand no paraffins were discovered
14 present in fermented tobacco. There have also been observed
15 a diminution of tritriacontane. Further analyses are
16 under way for the perfection of the methods of separation
17 and of the determination of said paraffins. Now, it speaks
18 about a table here which cannot be put on the screen.

19 In table 2 are reported
20 in percentage the compositions of tobacco and tobacco smoke
21 paraffins. In determining the composition of cigarette
22 smoke wax, we deducted the area due to the smeared background.
23 With a certain approximation the percentage of the iso-
24 paraffins was also determined in respect to n-paraffin for
25 the peaks corresponding to C₂₉ - C₃₁ and C₃₃, that is,

respectively 16 percent, 22.2 percent and 24.5 percent.

Conclusions. This work has demonstrated that high temperature gas-liquid chromatography can be used particularly in the search and determination of paraffins of tobacco and tobacco smoke; that paraffinic hydrocarbons at a low number of carbon atoms as well as considerable quantities of iso-paraffins are present; and that the complete analysis is rather rapid and it eliminates any fraction of paraffin waxes for their determination.

I have here also a part of another paper which it was first thought might be read, but only a paragraph or two Professor Carugno thinks may be of interest for those who have not had the opportunity to read it. I shall read those few lines now.

We have also published in Volume 692 of the Review Tobacco, January, 1959, the possibility of applying high temperature gas chromatography for the determination of some polynuclear aromatic hydrocarbons. Our inquiry was limited, however, to focussing our attention to the analysis procedure used with pure products, in order to discover the best conditions for separating and determining these substances.

The columns of Apieson L
on celite of various lengths and in different concentrations
of the stationary phase, at the temperatures of 230-250 and

1 260°C have been used. It has been possible to separate
2 and to mix the following polynuclear hydrocarbons: naphthalene,
3 anthracene, azulene, acenaphthene, fluorene, anthracene,
4 phenanthrene, fluoranthene, pyrene, 3-methylpyrene, 1-2 benz-
5 pyrene, perylene, 1-2-3-6 dibenzo-anthracene, anthanthrene,
6 3,4-9-10 dibenzopyrene. Isn't that a list for you? It sounds
7 like a lady putting ingredients in a good dish of spaghetti.
8 It takes skill.

9 We have indicated in this
10 study the retention times of the hydrocarbons which have
11 been examined.

12 We are conscious that
13 this method for determining the polynuclear hydrocarbons in
14 the smoke condensate presents some difficulties, not only
15 for the sensibility of detection, but also for the unreliability
16 of the basic line which probably is influenced by extraneous
17 substances. However, we decided to continue the experiments
18 with this method, and to use also an apparatus of gas
19 chromatography with hydrogen flame ionisation detector.
20

21 This is the part which was
22 to be read. Thank you.

23 CHAIRMAN SANICKI: I guess now Dr. Marni is
24 a finished speaker in chemistry and gas chromatography as well
25 as on Shakespeare.

Do we have any questions or

1 comments? Dr. Hoffmann?

2 DR. HOFFMANN: Hoffmann. Sloan-Kettering
3 Institute, New York City. We have done similar studies, but
4 we have never succeeded so wonderfully in gas chromatography
5 separation of paraffins or we are just in the very beginning,
6 but one work I would like to say; it might be a little
7 disappointing in our hands paraffins, long chain paraffins,
8 and when you show one slide, you show one thing and we may
9 have just the opposite effect, not promoting or carcinogenic
10 effect, we have a retardation effect. In other words, it
11 limits the resorption in our animal through -- which is mouse skin
12 of carcinogenic hydrocarbons, the resorption through the skin,
13 so that we get a decrease in activity. Can I show this one
14 slide?

15 CHAIRMAN SAWICKI: Yes.

16 DR. HOFFMANN: The light curve -- percent-
17 tumor-months diagram. The light curve shows bens(a)pyrene
18 in .005 percent concentration applied three times weekly.
19 "W" represents tobacco waxes, waxes from tobacco smoke
20 condensate with a melting point which varies a little, 56 to
21 58, 60 and you see practically no tumor response. I think
22 one mouse out of thirty got one tumor, and the middle curve
23 indicates very clearly the one we applied -- now, I have
24 to say we have not applied the tobacco wax, tobacco smoke
25 condensate waxes at the same time; this we have done Tuesday
and Thursday and two times weekly and benspyrene as in the

1 control, Monday, Wednesday and Friday because we had the
2 same idea to have with tobacco smoke condensate waxes
3 promoter, but it turned out in our hands just the opposite.
4 The tumor activity of benz(a)pyrene, I think, has indicated
5 here a significant decrease. Thank you very much.

6 CHAIRMAN SAWICKI: Any other questions or
7 comments? Frank Fox?

8 MR. FOX: Frank Fox, Public Health
9 Service, Cincinnati. I would like to ask the author if he
10 used any other substrates other than Apieson L for the
11 separation of the paraffins especially in the non-polar
12 synthesis such as SE-30 or other gum rubbers.

13 CHAIRMAN SAWICKI: Frank, rephrase your
14 question very simply so that --

15 MR. FOX: Were any other substrates
16 used for the separation of the paraffins?

17 CHAIRMAN SAWICKI: Any other substrates.

18 PROF. MARNI: He says only the smoke.
19 Does that make sense? He said no. Will you rephrase your
20 question, please?

21 CHAIRMAN SAWICKI: Stationary phase. Did he
22 use anything else besides Apieson L?

23 PROF. MARNI: He used only Apieson.

24 CHAIRMAN SAWICKI: Dr. Lyons?

25 DR. LYONS: I think preliminary purification

1 of the wax mixture can be obtained by forming adducts with
2 T-O urea (phonetic), it forms crystalline adducts, I think,
3 so it could be used along with the crystallization from
4 atrinal (phonetic) at -50.

5 CHAIRMAN SAWICKI: Dr. Hoffmann?

6 DR. HOFFMANN: Hoffmann. Sloan-Kettering
7 Institute, New York City. Dr. Lyons, is it not the absorption
8 how molecular -- molecules are formed -- I don't know the
9 exact name -- only with iso-paraffins and not with paraffins
10 when you take T-O urea (phonetic), so this is the way to
11 separate iso from normal? Thank you very much.

12 DR. LYONS: Yes.

13 CHAIRMAN SAWICKI: Any other questions or
14 comments? Thank you, Dr. Marni. I think you did a very good
15 job.

16
17 The next speaker will be
18 Dr. Fernand Dupire from the University of Louvain. He obtained
19 his Ph.D. in 1945. He has worked in the high temperature
20 gas chromatography of polycyclic aromatic hydrocarbons in tar
21 oils, road tars, et cetera. He has done quite a bit of work
22 in this field and I think he has something interesting here
23 that we can learn something from. Mrs. Bourgeois is
24 the interpreter and she will be sitting there ready to handle
25 any questions. Dr. DuFire.

DR. DUPIRE: